Vol. 17 No. 2

February, 1951

THE BOTANICAL REVIEW

Interpreting Botanical Progress

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Published Monthly except August and September at
111 E. Chestnut St., Lancaster, Pa.

Entered as second-class mail matter February 4, 1935, at the Post Office at Lancaster, Pennsylvania, under the Act of March 3, 1879.

THE BOTANICAL REVIEW

Founded in 1935 by
HENRY ALLAN GLEASON and EDMUND H. FULLING
Managed, edited and published at
The New York Botanical Garden
by
EDMUND H. FULLING

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Articles are obtained primarily by invitation. Unsolicited manuscripts are also welcome. In all cases, however, the editor reserves the right to accept, reject or suggest revisions in submitted material. All manuscripts should be on double-space typewritten pages, with references and citations in accordance with the customary style of THE BOTANICAL REVIEW.

Missing numbers can not be supplied unless notice is received within two months after appearance of issues concerned.

All correspondence should be addressed to THE BOTANICAL REVIEW, at 111 E. Chestnut St., Lancaster, Pa., or Fordham P. O., New York, N. Y.

THE BOTANICAL REVIEW

VOL. XVII FEBRUARY, 1951 No. 2

MITOCHONDRIA IN PLANTS. II[1]

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INTRODUCTION

Without minimizing the importance of the papers published during the past ten years on the subject of mitochondria in plants, it must be said that most of them are corroborative of previous work and suggestive in nature rather than definitive in any sense of the term. A few new interpretations have been made and some new techniques applied, but in general, the subject is still weighted with the incubus of an enormous early literature of confusing terminology and unsupported generalization which, by sheer repetition, have often achieved the status of dogma, and theories have far outstripped experimental evidence. It seems safe to say that there are at present no grounds for an attitude of poised assurance toward any of the theories put forward thus far concerning the nature and function of mitochondria in plants.

While it is doubtless an exaggeration to suggest that the solution of the problem of the functional significance of mitochondria will reveal the clues to *all* hidden problems or provide answers to *all* the questions concerning metabolism and heredity in the living organism, it is a truism to say that the problem *does* constitute one of the most important arcana in modern biology, and the cellular processes necessary for life will never be fully understood until the functional significance of mitochondria is revealed.

Recent findings in the fields of non-Mendelian heredity, viruses, enzymology and neoplasms have given a new urgency to the problem and have attracted many new investigators to the field. Thus the progress of the past decade, largely, however, by animal cytologists, but with techniques which seem equally applicable to plant cells, gives promise of an early solution to some of the questions about the function of mitochondria in the cellular economy.

[1] Supplement to article in The Botanical Review **6**: 85–147. 1940.

Since the mitochondria of plant and animal cells are generally regarded as homologous structures, it would seem that any new experimental technique or findings, whether based upon work with plant or animal tissues, would be of use and value to the plant cytologist. For this reason, some of the experimental work done by animal cytologists will be included in the present review. It might also serve to help break down the too rigid dichotomy existing between the sister disciplines.

To implement such a rapprochement, the reviewer would like to reiterate his plea (106) for a more definite nomenclature and for reduction to synonymy of the many terms occurring in the literature with reference to mitochondria in both plant and animal cells. It is proposed that the term "mitochondria" (*mitos*: thread; *chondrion*: small grain; singular: mitochondrion), coined by Benda in 1897, be retained exclusively on the grounds of priority, etymology, common usage and adequacy for our present knowledge of the subject. The term "mitosome", therefore, would logically refer to the thread- or rod-shaped mitochondria, and the term "chondriosome", to the granular forms. The term "mitochondriome" could refer, in a collective sense, to all the mitochondria in a cell or tissue. When in doubt that all the granules observed in the cytoplasm are truly mitochondrial in nature, the term "cytochondria", as used by Opie (109), might well be employed.

This terminology is at variance with that of Guilliermond and the French school who have written so many papers on mitochondria in plants, and may, therefore, need some defense. Guilliermond (71) uses the terms "chondriosomes", "chondrioconts" and "mitochondria" for our proposed mitochondria, mitosomes and chondriosomes, respectively. Historically each of these terms was borrowed from Meves, Lewitsky and Benda, respectively, and, since they are etymologically arbitrary and incorrect, the terminology suggested in this review seems preferable from the standpoints of priority, etymology and simple clarity.

In plant cells the terms "proplastids" and "archiplasts" are generally accepted as referring to those elements of mitochondrial morphology which show evidence of starch or other secretions, and which, with the plastids, comprise the plastidome.

The literature of the past ten years will be divided into two

categories with appropriate subdivisions for the convenience of review. These categories are the conventional and the experimental. By the conventional is meant those investigations using orthodox or modified techniques for the study of the morphology, distribution or histo-chemistry of mitochondria in fixed or living cells. By the experimental is meant those investigations using techniques largely physico-chemical in nature, some of which are not thoroughly standardized but are extremely promising, such as the isolation of mitochondria by maceration and ultracentrifugation from living cells. The dichotomy between these categories is not always sharp, nor are the points of view mutually exclusive.

REVIEW OF LITERATURE

Conventional

Status in the Cell. That plastids in plants can and do originate from mitochondria was perhaps best demonstrated by Lewitsky in 1911 (89). That all the mitochondria in meristematic cells are not involved in plastid formation can be determined in mature cells where functional plastids, which are self-propagating, often exhibiting a considerable degree of autonomy, and mitochondria exist together in the same cell. The number of mitochondria, it is generally observed, is always less than in meristematic cells, but since the number of plastids in any given cell is relatively inconsiderable, the reduction in number of mitochondria can not be ascribed entirely to plastid formation. That mitochondria arise from pre-existing mitochondria by division and do not arise *de novo* is generally accepted. What, then, is the explanation for their reduction in number in mature cells? Without attempting to ascribe physiological functions from morphological studies, it would appear that, since they are more numerous in meristematic cells which have a higher metabolic rate than in mature cells which are more specialized in function, their numbers are somehow associated with metabolic activity. That they have a total surface area in some cells larger than the nucleus and plasma membrane, with all that this implies in energy relations for the cell; that they are, at the same time, discrete bodies, separated in space by cytoplasm offering some degree of localization for reactions, may, and perhaps must, be of some significance. What, then, is their status

in the cell? Guilliermond (71) says that "it has been proved by our research that chondriosomes exist permanently in all phanerogam cells and that they are transmitted by division from one cell to the next". Other investigators, however, variously report the behavior of mitochondria during cell division, and further progress in this direction is blocked by the lack of a suitable technique for study of the behavior of mitochondria during cell division. Claude (40) also gives them the rank of *sui generis* structures, whereas De Robertis *et al.* (53) regard them as coacervates. Cowdry (44) states that they are widely distributed in plants and animals but not omnipresent in living protoplasm, and Bensley (12) reports that "these structures are in no sense permanent organelles of the protoplasm, but in case of need may be utilized for the nourishment of the cell . . .". This statement is supported by the reported disappearance of mitochondria during prolonged inanition and their rapid reappearance after feeding. The reduction in numbers of mitochondria during starvation and their increase with nutrition have been frequently reported by other investigators.

Considerations of technique do not, perhaps, permit the statement that some cells do not contain mitochondria, but it is obvious that their numbers fluctuate widely in different cells and tissues and with the nutritional levels. However, since plastids also fluctuate in numbers which vary with the topography, environment and the morphogenesis of the plant, the argument against accepting mitochondria as cell organs *sui generis* would seem no more convincing than that for the plastid which is generally so considered. If mitochondria are cell organs *sui generis* in plants, it would seem that they must be so considered in animals, or theories regarding their homology must be revised. This is both logically and experimentally unnecessary. The suggestion (153) that mitochondria may not be the ultimate self-propagating morphologically definable unit of the cell but may be in turn derived from smaller units—the microsomes or other ultracentrifugable particles—may shed some light on their apparent disappearance or absence from some cells. Mitochondria may thus either revert to the smaller forms or be utilized metabolically. In either case their *sui generis* nature would remain unchanged.

The cytochemical demonstration of ribonucelic acid in mito-

chondria of plant and animal cells during the last decade makes relevant, in this connection, the following quotation (127): "Combined cytochemical and genetic techniques and the identification of viruses as nucleoprotein in constitution make it seem probable that cellular structures containing nucleic acid possess genetic continuity".

Theory of the Dual Nature of Mitochondria in Plants. The theory that there are two types of mitochondria in plants, functionally and genetically distinct, albeit apparently identical physically and chemically, was tacitly or admittedly advanced because of the assumed compulsion of considerations of homology. These were based upon the fact that, although both plant and animal cells contain mitochondria, some of the mitochondria in plant cells are observed to develop into plastids, whereas animal cells, containing no plastids, show no similar development of the mitochondria. Guilliermond originated the theory in 1920 and reiterated it in many subsequent papers (for review see 70, 71, 106). He called the mitochondria in plants which developed into plastids, the active mitochondria, and those which did not, the inactive mitochondria, and the latter were the homologues of the mitochondria in fungal and animal cells. The cytologic evidence for this theory is most meager and unconvincing, and, if freed from the assumed compulsion of the requirements of homology, would collapse. In view of recent developments, the theory is of only academic interest.

Bowen, an animal cytologist, proposed a similar theory based again on the assumed requirements of homology, but rejected Guilliermond's advocacy as "a matter of words". Bowen, however, did support his theory with cytological evidence. The evidence consisted largely of the topographic distribution of two different morphologic forms of mitochondria and their behavior during cell division. Both Guilliermond's and Bowen's work and theories were recently reinvestigated (106) and could not be corroborated. Cowdry (44) long ago saw no need for the theory. The influence of Guilliermond and Bowen has been considerable upon the literature and attitudes of subsequent investigators, and it would be an exaggeration to say that the theory is dead. But the history of the subject is also replete with biased reviews in which evidence against the theory of the duality of mitochondria

has been either omitted or cavalierly treated, and it is felt by the present reviewer that in this way the theory has enjoyed a prominence all out of proportion to its intrinsic validity.

Perhaps the most impressive support for the theory comes from the work of Sorokin (131) on living cells stained supra-vitally with Janus Green B. She claims that mitochondria which form plastids can be differentiated from those which do not by the fact that the latter alone stain with Janus Green B. Anderson (3) could not corroborate Sorokin's results, and Guilliermond (71, p. 103) says Janus Green stains both mitochondria and leucoplasts, but conceded that "under certain conditions Janus Green stains the chondriosomes more intensely than the leucoplasts". Sorokin also reported reduction by mitochondria of Janus Green to a rose-colored derivative and thence to its leucobase, and the reaction was reversible in *Elodea,* tulip and *Narcissus.* Guilliermond (71, p. 67) stated that "the vital staining of the chondriosomes by Janus Green is only transitory. It is observed that at the end of a few moments the chondriosomes lose their green color, whereas a rose tint appears in the vacuolar system. These observations are explained by the fact that the chondriosomes reduce Janus Green to its rose derivative and this latter, having more affinity than its oxidized form for the vacuolar system, diffuses into it". And (71, p. 120) he continues, in refuting Joyet-Lavergne, by saying, "In fact, we shall see that the chondriosomes do not seem to have of themselves any reducing power. In any case, they are incapable of reducing Janus Green to its leucoderivative, contrary to what has been thought up to now". Strugger (134), using phase contrast, saw no distinction between mitochondria in plant cells.

It appears almost incredible that the status of Janus Green B as a vital stain for mitochondria in plants has not been thoroughly established. It is generally conceded that it specifically stains the mitochondria of animal cells, but its efficacy for plant cells is still sharply debated and there is an equivalence of testimony for and against its use (104, p. 97). The work of Opie (109, 110) may partially explain the confusion. Working with animal cells, he observed that some of the cytoplasmic particles stained with Janus Green and some did not, and that granules which were indubitably mitochondria lost their stainability in hypotonic solu-

tions and in the presence of certain solvents. According to Opie (110), "Water promptly removes from the cytoplasm both the basophil material that gives the reactions of ribonucleic acid and the material that gives to the mitochondria their characteristic stain". Pfeiffer (113) assumes that Janus Green stains the mitochondria in plants; Woods and Du Buy (141) used it; and, whereas one may differ with Sorokin in her interpretations of the significance of structures stained with it, one can not doubt that Sorokin was successful in staining mitochondria *in vivo* with Janus Green B. The reviewer has never been successful with it and, since one is reluctant to admit to technical ineptitude, he is inclined to place the blame upon the water supply. Chlorinated water, though twice distilled and rendered approximately isotonic with either sugar or salts, will not, in my hands, produce clearly stained mitochondria with Janus Green B.

However, it should be recalled that the main support of the theory is derived from fixed and stained preparations. Much of Guilliermond's evidence was derived from studies on *Elodea* fixed in Regaud's fluid. He stated (71), "It has been seen that among the elements which constitute the chondriome in meristematic cells of the bud of *Elodea canadensis,* it is possible to distinguish between the chondrioconts [rod-shaped mitochondria] which become chloroplasts during cellular differentiation, and the granular mitochondria which do not participate in this phenomenon but elongate into rods and later into chondrioconts". One assumes this to mean that the granular mitochondria elongate to form "chondrioconts" but that development of these "chondrioconts" ceases there, whereas the "chondrioconts" which form plastids, although identical in morphology, not only can be distinguished from those originating from granular mitochondria, but also are genetically distinct and of a different chemical constitution. Regarding the latter he stated, "It is obvious that the two categories must, after all, possess differences in chemical constitution. Otherwise it could not be explained why one has functions which the other does not have" (71, p. 113).

Historically, the controversy originated from the researches of Pensa (111) and Lewitsky (88) in 1910. These investigators independently (Pensa in July and September; Lewitsky in December) discovered and described the origin of plastids from mito-

chondria. A storm of protest arose from the Schimper-Meyer school which maintained that plastids always arose from pre-existing plastids. This opposition persisted despite the fact that the Schimper-Meyer faction offered no new evidence, whereas Lewitsky in 1911 (89) presented excellent photographs of plastid formation in both living and fixed cells of *Elodea*. To the plastid-forming mitochondria he gave the name "chondriokonts", a term later used by Guilliermond. Guilliermond's preoccupation with his duality theory is traceable as far back as 1912, when he attempted to compromise the differences between the two schools by suggesting that plastid formation does not afford a sufficiently exact definition of the function of mitochondria and attempted to distinguish between young plastids and mitochondria by their different solubilities in alcohol and acetic acid.

It has been known for some time, however, that plastid development from a mitochondrial origin involves an increase of the protein moiety of the mitochondrion which renders it less and less soluble in fat solvents with growth.

Since it is a frequent practice of contemporary investigators to credit Guilliermond with the discovery of the mitochondrial origin of plastids, it should be reiterated here that both Pensa and Lewitsky independently antedated Guilliermond in this respect. Although Pensa's paper appeared in print several months prior to Lewitsky's, the latter's work was more comprehensive and definitive—in fact, it can be said that it has not been improved upon—and it seems that Lewitsky should receive the major credit.

Perhaps the theory of the duality of mitochondria has been excessively belabored here, but the reviewer would like to see it quietly interred and the subject relieved of a barren hypothesis. For those who feel that the requirements of homology still demand it, perhaps the recent findings of mitochondria in animal cells secreting fat (136, 137), developing into yolk bodies (50, 60, 61) or secretory granules (36) will suffice. McCurdy (95) finds that the form of mitochondria varies with nutrition, i.e., in the liver of well fed adults of *Fundulus* the mitochondria are predominantly elongated, whereas during starvation they assume a granular form. Thus their shape is correlated with the amounts of fat and glycogen present. And Worley (147) finds difficulty in distinguishing between mitochondria and Golgi bodies in stained

fixed material, but (148) claims to differentiate them by vital stains in animal tissue. Pollister (114), from tissue culture studies, suggests that only the rod-like mitochondria are normal and that the granules and short filaments are largely artifacts. At the other extreme, Noel *et al.* (107) describe three different types of mitochondria in the three different types of cells in the anterior lobe of the hypophysis of the white mouse. Ceruti (31, 32) claims to have isolated three types of mitochondria from the prothallial cells of *Ginkgo,* and O'Brien (108) described three kinds of mitochondria in germinating seeds. Thus it seems clear that it is unwise to conceive too narrowly or to accept too uncritically any theory, however plausible, from either morphological studies alone or considerations of homology when the evidence, which could be greatly multiplied, is so clearly indicative of only a protean nature.

Of the five categories of cytoplasmic bodies described by Bowen in the cytoplasm of plant cells, the plastidome and pseudochondriome corresponded with Guilliermond's active and inactive chondriome, respectively. The distinctions of Bowen were based upon fixed and stained material, and the error seems to lie in the excessive faith in the image so presented. Technical modifications were shown to produce almost any desired image of a cell, and neither the distinction between plastidome and pseudochondriome nor the validity of the osmiophilic platelets as cell organs *sui generis* can be accepted at the present time (106).

Among the other published papers of the past ten years, one can cite the following as explicitly or implicitly supporting the theory of the dual nature of mitochondria: Bauer (8), working with liverworts; Dangeard and Eymé (46), in mosses; Jones (85), on *Elodea* and *Triticum;* O'Brien (108), who implies a dual nature in mitochondria from his observations on the germinating seeds of *Triticum* and *Secale;* Rezende-Pinto (117), by the use of an acetic acid-containing fixative and a tannin-iron preparation which he says stains the mitochondria but not plastids; the studies of Roberts and Southwick (122) with the electron microscope in which they described the origin of chromoplasts from small colored bodies one micron or less in diameter; Sorokin's (131) work which has been mentioned; Buvat's (24, 25, 26, 27) studies on chicory, in which the mitochondria are separated from leucoplasts

on the grounds that the former became hypertrophied in water, whereas the latter did not; Yin (150), who found phosphorylase activity in chloroplasts, chromoplasts and leucoplasts, but not in mitochondria; and Yuasa (151), who separated them in the ferns on the basis of their staining with Janus Green.

Among the papers of the past ten years one can cite the following which do not support the theory of mitochondrial duality: Anderson's (3) work on living and killed tissues of lily; Claude's (36) support of Noel and his homologizing the secretory granules of animal cells with plastids, since both are of mitochondrial origin; the similar work of Das (50) who described the origin of albuminous yolk bodies in an animal from a mitochondrial origin; Gonçalves da Cunha's (64, 65, 66) refutation of Guilliermond's theory; Marengo's (93) work on a fern in which he described the reversion of plastids to, and their subsequent origin from, mitochondria; McDonough's (96) and Ritchie's (120) studies on fungi, in which mitochondria similar in pleomorphism to higher plants were described; Newcomer's (106) specific criticism of the theory; Strugger's work (134) with phase contrast; and the paper of Takagi (135).

One might, with equal justification, postulate a similar dual nature to the mitochondria in animal cells, for a similar morphological transformation has been observed of the mitochondria into glycogen granules, yolk bodies, fat droplets and numerous other secretory products. In plants they have been similarly observed to develop into oleoplasts which secrete oils and fats; others develop into leucoplasts which can change hexose sugars into starch, whereas in many monocotyledons the plastids have either lost or never had the ability of starch synthesis. And consider the successive developments of a mitochondrion into a leucoplast, chloroplast and finally a chromoplast, as can be observed in the tomato, which involves profound changes in function, chemistry and morphology. If these versatile mitochondria in plants and animals could be separated by morphology from those which show no morphological development, grounds for the dual nature of mitochondria might be suggested. But the techniques used thus far for such a demonstration are so capricious and subjective in nature as to be worthless, and the polymorphism of mitochondria has been so clearly established in living and fixed cells that morpho-

logical methods seem to be futile for the elucidation of functional distinctions.

Genetic evidence would also seem to negate the validity or necessity of such a theory. The metabolism and, to a considerable extent, the morphology and behavior of the cellular constituents are conditioned by genetic constitution. In corn there are at least a hundred known instances of genic factors modifying the chlorophyll condition of the plastids from albinism to degrees of virescence. The morphology of the plastids is similarly modified throughout the gamut of changes. Similarly, the fact that the fungi contain mitochondria but not plastids does not necessitate the homologizing of these mitochondria with those of animal cells exclusively. Many of the higher seed plants are also fungal in metabolism, ranging from parasitism to saprophytism (cf. 106). Since these plants achieved their fungal metabolism at obviously a later date than did the fungi, it seems clear that the metabolic differentiation was caused by genetic change or mutation. Thus the *a priori* concepts upon which the duality theory is based do not hold, and a simpler explanation which fits the evidence seems to suggest that the mitochondria of plants and animals, while homologous structures, are functionally versatile in response to the genetic constitution and metabolic requirements of the organism. This is essentially the eclectosome hypothesis of Regaud.

Techniques. The electron microscope, improvements and general availability of the phase microscope, and new cytochemical techniques, while almost entirely responsible for the considerable advances in our understanding of the functional significance of mitochondria in the past ten years, do not obviate the necessity for improvements in morphological techniques for intracellular study. Certain desiderata, such as the type of tissue applicable and phase of development, attend these new experimental techniques in addition to the elementary but important consideration that, in general, no permanent records of the original preparation are left for subsequent examination and comparison. Furthermore, a good controlled cytological technique, like art, permits exaggeration of certain aspects of the image presented, such as the pre-treatment of chromosomes to study coiling, which is of great importance and of course requires no apology. A well-fixed and stained preparation, in addition to being a pleasure to behold, presents the cellu-

lar constituents as a whole and thus permits a relational comparison which not only is valid in itself, but is the spadework for more refined, but derived, experimental technical studies of the individual units of the cell.

The reviewer does not, therefore, entirely agree with the statement (53) that, "We can affirm that in this case, as in many other aspects of cellular life, the possibilities of the morphological method have been practically exhausted and that one must hope for the development of new cytochemical methods to solve entirely the problem of the functional significance of the chondriome". Cytology is still primarily a visual science, and morphological methods are still necessary to corroborate cytochemical findings as well as to suggest new attacks upon the problem. The prevailing barrenness of the morphological method might better be ascribed to the fact that for the past fifty years, with few exceptions, the same killing fluids and techniques have been used with little or no modification. As more is learned from cytochemical techniques it is hoped that morphological methods will improve to the point where it will be possible to produce a killed and stained cell which bears a complete, or nearly complete, similarity to that cell in a living condition, with the advantage of greater visibility of its parts. The problem of combining the hitherto mutually exclusive acid and basic fixation images of the cell, though urgent, has been largely overlooked (106).

Morphological studies of the cell have reached their present nadir, and deservedly so, largely because investigators have used the techniques as recipes and have accepted the images thus presented as precisely correspondent to the living cell, and have minutely described structures and structural modifications which may have been artifacts. And the so-called vital techniques are not immune from this criticism, as the record proves. It is felt by the reviewer that the search for new chemicals for fixation, rather than the modification of existing formulae, associated with a consideration of the chemical properties of the cellular constituents as revealed by cytochemical techniques, is necessary for definitive morphological studies of mitochondria. Little has been done in this direction thus far. There is little literature in this field, but a perusal of the books by Mann (92) and Baker (5) yields many helpful suggestions and warnings.

FIXATION. Opie (109) used a mixture of lanthanum acetate and formalin, and Newcomer (106), following a suggestion of Baker, incorporated quinone in Zirkle's fixative, for mitochondria. The latter fluid occasionally produced beautiful preparations, preserving both the nuclear and cytoplasmic constituents of the cell, but the technique, despite many efforts, has not been standardized and is capricious. Petrunkevitch (112) produced a fixing formula for mitochondria in insects, which is a contradiction of all previous assumptions in that it contains fat solvents and is extremely acid (pH 1.35). It is a bromophenol-cupric fixing fluid, and the reviewer has found no report of its having been used by other investigators. Ritchie (120) made a comparative study of the effects of seven different fixatives on *Russula,* and Newcomer (106) tried every killing fluid found in the literature of mitochondria as well as some modifications of many of them (a total of 189) without achieving much new information, save, in the latter study, evidence for the artifact nature of the osmiophilic platelets, and the general unreliability of many techniques which were reported to preserve the cell in a life-like condition, and the negation of the theory of the dual nature of mitochondria in plants.

STAINING. Cain (28) and Dry (55) have presented modifications of the aniline-fuchsin technique which they claim to be superior, and Harman (74) has introduced an aniline-fast green (hot) combination for mitochondria in animal cells. Rezende-Pinto (117) used a tannin-iron preparation, and Newcomer (105) modified the Kolatchev technique and blackened mitochondria selectively with osmic acid.

Sorokin (131) described in detail the use of Janus Green B for vital staining, and Joyet-Lavergne (86) presented a new technique for vital staining of the mitochondria with indigosols which are stable leuco-derivatives of indigo dyes and which require oxidation for color. Monné (101) has described the use of 32 basic dyes for the selective staining of mitochondria supravitally. Hall (73) reviewed five papers dealing with mitochondria in the flagellates, and Hollande (79) has described them in some protozoa.

ELECTRON MICROSCOPY. Buchholz (23), Porter, Claude and Fullam (115) and von Mühlethaler, Miller and Zollinger (103)

have described the preparation of tissues for the electron microscopy of mitochondria. Buchholz used plant tissues, which are very difficult, and the other investigators, animal tissues. The hazards and limitations of electron microscopy are considerable, and suspensions of material previously isolated by maceration and ultracentrifugation appear to offer some advantages. The technical innovations by Porter *et al.* (115) resulted in superior preparations.

Experimental

Structure and Chemistry. The pleomorphism of mitochondria is proverbial. Buvat (24–27) described their reversible pleomorphism in tissue cultures of plants, and Meitres (98) suggested that they are a heterogeneous composite of small granules united into chains. Evidence of spiraled internal differentiation based upon fixed material was suggested by Newcomer (106) and later supported by Buchholz's (23) studies with an electron microscope. Zollinger (153), with the aid of phase microscopy, examined living animal tissues and described rod-like mitochondria with bulbous or vesiculated ends, storage granules of mitochondrial proportions and smaller bodies designated "microsomes" which he suggested constituted the mitochondrial primordia. Claude and Fullam (41) made an electron microscope study of mitochondria isolated in a purified state from neoplastic animal tissues by mincing and differential centrifugation, and described spherical bodies 0.6 to 1.3 micra in diameter, surrounded by what appeared to be a differentiated membrane. Smaller granules were imbedded within the mitochondrial bodies.

A later study by Porter, Claude and Fullam (115) of tissue culture cells by electron microscopy and an improved technique of mounting clearly demonstrated filamentous, spherical or vesicular mitochondria with inclusions in the form of masses of greater density along the sides, which might have been inorganic salts or reduced osmium.

Opie (109, 110) stated, "A study of the cytoplasm of cells of normal organs and of tumors has given evidence that it consists in great part of discrete particles, cytochondria, with surface properties that make them permeable to water and to other substances. They have an outer rim that takes up stains and a clearer or unstained center. . . . Of the bodies that I have designated cyto-

chondria [cell grains], only a part have the characteristic staining reaction of mitochondria". The cytochondria were found to constitute an osmotic system within the cytoplasm of cells, corroborating the previous suggestions of the presence of a mitochondrial membrane. And further support for the presence of a membrane was given by von Mühlethaler *et al.* (103) from electron microscope studies [2].

Positive birefringence of mitochondria is reported by Monné (102), contrary to the findings of most investigators. De Robertis *et al.* (53) regard them as coacervates, whereas Ceruti (31), because of their assumed structural complexity, says they must be considered as living and not as coacervates.

Prior to the past decade, the chemical properties of mitochondria in plant cells were intimated by indirect techniques, such as solubilities by fat solvents, melting points and resistance to acid fixation. These results were only grossly qualitative, and how much of the reaction was due to the reagent and to what extent it was due to a general cytolytic cellular response could never be determined. However, the protein-lipoid complex of mitochondria was clearly indicated.

In 1934 Bensley and Hoerr (13) originated a technique by which they claimed to have isolated mitochondria in purified fractions adequate for a quantitative chemical analysis. The technique consisted of comminuting either fresh or frozen-dried material in an isotonic (0.85%) salt solution through bolting silk and by subsequent centrifugation isolating the mitochondria on the basis of specific gravity. The recovered pellet could then be analyzed chemically, and the following percentage composition found in the dried mitochondria of guinea pig liver:

Proteins and unknowns	64.67
Glycerides	28.88
Lecithin, cephalin, etc	4.2
Cholesterol	2.25

Baker (4) suggested that the 4.2% of lipines would be increased if better tests were applied to the large fraction of fatty material that is not precipitated by acetone in Bensley's technique. This was apparently later verified by Claude (39) who segregated and purified certain elements of the cell by differential sedimenta-

[2] See footnote 4.

tion in a centrifuge. The extract was fractionated into three portions: mitochondria, submicroscopic particles or microsomes, and constituents of relatively low molecular weight. The elementary composition of the thus purified mitochondria was:

11.5%	N
1.6	P
27.0	lipids

He further reported that the pH and nitrogen content of the lipid portion suggested that as much as 75–80% of the lipids of mitochondria was represented by phospho-lipids. Tests for ribose nucleic acid were positive, and biochemical tests for oxidative enzymes gave the following values:

For succinoxidase, QO_2 equals approximately 135 to 208
For cytochrome oxidase, QO_2 equals approximately 500

This technique has recently been modified and utilized by a number of investigators, and constitutes perhaps one of the most valuable innovations in mitochondrial investigations (9, 11, 17, 18, 19, 37, 39, 40, 75). The technique, however, has not produced the same results among its practitioners (cf. 10, 37, 75), and there have been some serious criticisms of it. Perhaps the most serious is based upon the difficulty of reconciling the known fragility of mitochondria with the maceration, mincing or comminution of the tissue, and some investigators have even used a Waring Blendor for mincing. Thus Cowdry (43, 44) warned that pinching the tissues slightly with forceps, or letting their surfaces dry, renders them useless for mitochondrial investigation, and, with most investigators, considered them the most delicate indicators of cellular injury. Since their response to injury usually resulted in their disappearance—as can be demonstrated by any morphological technique—it is difficult to see how they survive crushing prior to centrifugation, even though bathed in an isotonic fluid. And Chambers (33) asks, on the basis of his extensive micrurgical studies, to what extent the several morphologically different components of the cell can be isolated without loss of their true nature. Von Haam and Schuh (138) concluded their study by stating that, "We feel, however, that his [Bensley's] method is too injurious to the shape of those small intraplasmatic structures to preserve them in a recognizable state". Since this

study also entailed the use of an electron microscope, part of the disagreement might well have been due to the technical difficulties involving spreading and drying the material for photography. The most specific and sweeping criticism of the technique has come from Danielli (47) who has the following to say about methods involving maceration prior to isolation: " In these procedures cells are disintegrated by various techniques, and the resulting mush is subjected to differential centrifuging, by which means fractions of different densities are obtained. It is claimed that almost pure nuclei and mitochondria are found in certain fractions, and on occasion it is further claimed that the chemical substances, including enzymes, found in these fractions, are both qualitatively and quantitatively identical with those present in the nuclei and mitochondria of the living cell. None of these claims has been established, and there are reasons for supposing that they are not justified. By microdissection methods Chambers and others have shown that when a nucleus is removed from a cell into a foreign environment, two things may occur: either the nucleus dissolves almost completely; or it sets into a rubbery gel. In neither case is its physical condition similar to that found in the living cell. . . . Both the nucleus and cytoplasm must be regarded as highly complex colloidal systems, the nature of which, and the distribution of substances between which, are likely to be profoundly altered by much milder changes than those involved in maceration. . . . It therefore cannot be assumed that because an enzyme is found in such isolated ' nuclei ' or ' mitochondrion ' this enzyme was in fact present in that structure in the living cell . . .".

That there is need for caution in the interpretation of results secured by such procedures is evident; that such caution is always exercised is not so obvious.

Barron (7) voiced a similar criticism, concluding by saying, " Hasty and unfounded applications of the results obtained with damaged cells and the theories built around such results to the chemical processes going on in the living cell have, alas!, crowded the literature and confused thought ".

The pertinence of these criticisms can not be determined at the present time, but the reviewer, and possibly other investigators of mitochondria, can take a more charitable view of the situation in the light of the sterile scholasticism previously prevailing in refer-

ence to mitochondria, which was perpetuated by old and inadequate techniques. Despite the apparent barbarity of the technique and the supposed fragility of mitochondria, maceration, followed by differential centrifugation, has yielded pellets which, upon microscopic examination, bear a good resemblance in size and form to mitochondria. The beautiful photographs by transmitted light taken by Schneider (125) from preparations produced by Hogeboom, Schneider and Pallade (78) of mitochondria so isolated leave little to be desired and are, if anything, superior to most orthodox morphological preparations. In addition, the mitochondria stained with Janus Green B at concentrations between 1 : 20,000 and 1 : 40,000, could be fixed in OsO_4 and stained with aniline-acid fuchsin. These cells were broken in a 30% sucrose solution, and it is possible that hypertonicity is desirable for their preservation (82). The work of Opie (110) regarding the osmotic property of mitochondria might also suggest it.

The work of Dounce (54) in devising a technique for the separation and isolation of intact nuclei from cells which can be identified morphologically and cytochemically with Feulgen should also be mentioned in this connection, and his suggestions of procedure in using the technique may also explain the failures of some investigators [3].

Kingsbury in 1912 first suggested that mitochondria are the seat of the respiratory enzymes, and Marston in 1923 produced evidence for their carrying proteolytic enzymes. In 1938 Claude (36, 37, 40) separated by differential centrifugation the following components of the cell in relation to the centrifugal pole: glycogen, mitochondria and secretory granules, small particles (microsomes), the " purple substance " and Golgi. The nucleus was at the level of mitochondria and zymogen granules but above the glycogen after 60 minutes at 18,000 $\times$ gravity. The term " microsome ", coined by Hanstein in 1880, is used by Claude to designate the small granules, 50–200 millimicra in diameter, and, though the microsomes are similar chemically to the large granules (0.5–2.0 micra in diameter) and possibly their precursors, they are separable chemically and physically from other cellular constituents. The mitochondrial and secretory granule fraction obtained by

[3] See also the paper by J. L. Still and E. H. Kaplan (Localization of Oxidases. Expt. Cell Res. **1**: 403–409. 1950) which appeared too late for inclusion in the bibliography of this review.

Claude was similar in physical characteristics to the mitochondrial fraction obtained by Bensley and Hoerr, but different in chemical analysis, which led Claude to suggest that it is probable that mitochondria had never been isolated in pure form and that the substance analyzed by Bensley and Hoerr corresponded with the fraction designated as secretory granules by him. Bensley (11) later conceded that his original preparations of hepatic mitochondria were contaminated with glycogen as were Claude's. Bensley confirmed the status of Claude's microsomes, being similar in composition but quantitatively different from mitochondria. Hoerr (75) considered the discrepancy between Claude's and Bensley's work to be due to terminology. Thus the secretory granules were really mitochondria, according to Hoerr, and the confusion arose because Claude relied too heavily upon the results of Noel who claimed that secretory granules arise from mitochondria, but whose work was based upon inadequate histological evidence and has not been confirmed. Hoerr says the differences between mitochondria and these undeniable secretion antecedents can be readily demonstrated by staining methods devised by Bensley, but Schneider demonstrated their homology (125).

Garrigues (62) described the action of colchicine and chloral hydrate upon the mitochondria of the root tips of *Vicia faba,* and Ryland (123) observed the effects of colchicine, indole acetic acid, cyanide and 2,4-D upon them in *Allium.* Structural modifications were noted in both cases save with colchicine to which they were very tolerant.

Beams (9), from his studies with an ultra-centrifuge, noted that the mitochondria of animal cells and bean root-tips possessed a specific gravity greater than the surrounding cytoplasm.

Function. In general, botanical investigations of mitochondria during the past ten years, besides being relatively few in number, have produced no new or corroboratively conclusive indications of their functional significance. Tentative assumptions of secretory functions based upon morphology and distribution in the cell, their role in plastid formation and considerations of their dual nature might be given as the contribution of botanical cytologists to the subject. No promising new morphological techniques have appeared; preoccupation with former theories, which in retrospect seem to be of only academic interest, has apparently produced a

general apathy toward the subject; and only the hardy few have remained interested. There are a few exceptions to this general picture (56, 57, 59, 141), whose work will be discussed later.

Morphological studies by animal cytologists have been just as meager in results. Secretory functions have been reiterated (60, 61, 95, 136, 137, 152 and others), and a function relative to mitosis, because of their orientation during cell division, has been suggested (34).

The real advances toward elucidation of the functional significance of mitochondria seem to be based upon the discovery by Bensley and Hoerr in 1934 (13) of a method for separation and isolation of mitochondria, upon improvement of the technique by Claude (36, 37) and others, the influence of Caspersson (30) and the contribution of Brachet (17, 18) of a cytochemical technique for the detection of ribonucleic acid in the cytoplasm, and the localization by Claude (for review see 40) of ribonucleic acid in the mitochondria and other cytoplasmic particles. Hoerr (75), Lazarow (87), Davidson (51), Zollinger (154) and others have since confirmed the ribonucleic acid content of mitochondria. Since nucleic acids of the ribose or thymus types have thus far always been associated with the property of self-duplication of the particle with which it is associated, the discovery of ribonucleic acid in mitochondria assumes special significance. The assumption that the simpler viruses consist of practically pure ribose-nucleoproteins, the relationship between viruses and neoplasms, and the isolation of ribonucleic acid by Menke (99) from green leaves, followed by its isolation by Woods and Du Buy from plastids, have led to a series of interesting and significant investigations, broadly conceived and experimentally executed, supported in turn by morphological studies which carry considerable conviction for several theories of their functional significance in both plants and animals.

In addition to ribonucleic acid, cytochemical techniques have corroborated the early suggestions of Kingsbury and Marston that mitochondria are the seat of the respiratory and hydrolytic enzymes (18, 19, 22, 76, 77, 78, 124, 125, 126). Thus amylase, succinoxidase, cytochrome oxidase, cytochrome C, adenosine-triphosphotase, 18 amino acids (91) and vitamin A (63) have all been identified on mitochondria.

There is not, however, complete agreement on the localization of ribonucleic acid and enzyme systems on the mitochondria. Thus Brachet (18) and Brachet and Jeener (21) identified them with the small granules or microsomes of Claude and do not think, from their own researches, that they can be yet identified with mitochondria. And Holter (80), Holter and Kopac (81) and Wilber (139) found that the intracellular enzymes studied by them were localized in the hyaloplasm and not in any formed bodies. Monné (102) reports ribonucleic acid, the respiratory and hydrolytic enzymes in structures which he calls " chromidia ", but which, from his definition, correspond to Claude's microsomes.

To the reviewer, the evidence of Claude and his associates (37–41, 76–78, 124–126), of Lazarow (87) and of Woods and Du Buy (144) for the localization of ribonucleic acid and the respiratory and hydrolytic enzyme systems on mitochondria is convincing. That they might also constitute an intracellular osmotic system, as suggested by Opie (110), for which he presented considerable experimental evidence, in addition to their other presumed functions, is not without plausibility [4].

A relationship between mitochondria and virus disease was suggested by Graffi (67, 68) in 1940. He believed that the cancerous processes initiated by the cancerogenic hydrocarbons are due to their effect upon the mitochondria by inducing irreversible and permanent changes which are transmissible to the following cell generations and which alter cellular behavior from normal to malignant. He also suggested that certain viruses, as the Rous type, are such altered mitochondria which, when introduced in normal cells, cause malignancy. Further evidence along this line was presented by Woods, Du Buy and their associates. In their early work (140), involving chloroplast derangement in leaves infected with tobacco mosaic virus, a possible connection between the virus protein and the chloroplast protein was suggested in which the tobacco mosaic virus protein might have been a derivative of the plastid protein. A similar idea was presented inde-

[4] In this connection, two papers (74*a*) which appeared after this review was completed should be consulted. Space limitation permits only a summary of the second part: "Mitochondria are visualized as composite structures, in part of which the integrated enzyme systems are concentrated. The behavior of mitochondria in deionized water and in salt solutions is compatible with a gel-like structure and does not require the presence of a semi-permeable membrane".

pendently by Blodgett (15) with reference to the peach calico virus. The previous work of Claude (35), who isolated the protein fraction from the mitochondria of cancerous animal tissues and obtained an elementary chemical analysis almost identical with certain plant viruses, supported the idea, and later Du Buy and Woods (56) obtained an ultra-violet absorption curve of plastid nucleoproteins similar to that obtained by Claude for the tumor and non-tumor fractions of mouse and avian cells and by Bawden (57) for bushy stunt virus. Thus a chemical relationship or spectrum was established between the nucleoproteins of plastids, mitochondria and viruses, which was also supported by the following morphological and genetic evidence (59, 141). According to these investigators (145), since mitochondria have a definite heredity and are capable of mutation, and since plastids are of mitochondrial origin, possessing similar hereditary properties, both of which are under control of the ribose-nucleoprotein component, a mutation of the mitochondrion would be reflected qualitatively and/or quantitatively in the resulting plastid and produce variegated areas of a periclinal, sectorial or mosaic-sectorial pattern in the plant. Histological examinations revealed a gamut of plastid abnormality from near-normal to mitochondrial in size and from light green to colorless. The extremely abnormal cells resembled those infected with "typical viruses". The intracellular systems were competitive in that the extreme variegation-inducing mitochondria inhibited normal plastid development in the same cell. In the most extreme cases the variegation-inducing agent was invisible and intercellularly transmissive, both by grafting and probably through plasmodesmata. They also reported (57, 144) intercellular mitochondrial migration and, in support, cited the work of Horning and Petrie who reported intercellular migration of mitochondria between the scutellum and endosperm in germinating cereals. Subsequent investigators by morphological methods (104, 108) failed to confirm the migration of mitochondria across the epithelial cells between scutellum and endosperm.

Billingham and Medewar (14), however, clearly demonstrated that in guinea pigs the black color, due to melanin granules, is invasive when grafts are made on white areas, and deduce that the blackening factor or some precursor of it is capable of self-multiplication and migration from cell to cell. This involves an infective

cellular transformation, i.e., a conversion of one true breeding type into cells of another true breeding type by a serially transmissible process which they likened to a virus infection.

As to the graft-transmissible variegations, Rhoades (119) was of the opinion that Woods and Du Buy had not shown that the variegations were not themselves due to a virus and that the intermediate stages of chloroplast breakdown which were observed in transition zones between green and colorless areas and which were attributed by Woods and Du Buy to the invasion of virus-like particles of mutant mitochondrial origin were paralleled by the conditions found in transition zones in iojap leaves of corn (118) which are determined by a Mendelian recessive gene, but which are cytoplasmically inherited.

From observations of gradual modifications over the gamut of variegation-inducing plastids, Du Buy and Woods (56) suggest the following parallelism between the morphological and chemical evidence for the evolution of viruses from plastid or mitochondrial ribose-nucleoproteins: (*a*) loss of part or all chlorophyll followed by further loss, including carotenoid pigments, resulting in colorless plastids; (*b*) loss of complex plastid structure, giving proplastids or mitochondria; and finally (*c*) the occurrence of small submitochondrial units which resemble slow-moving viruses, such as those causing *Abutilon* mosaic. This morphological degeneration is paralleled by the breakdown of the chemical linkage of the chromoprotein-lipoid plastid complex to the free virus nucleoprotein and changes in the oxidizing enzyme systems. It is suggested that animal viruses (56) of the ribose-nucleoprotein type could similarly be derived from the mitochondriome, and in this way a connection might be established between certain cancer agents and viruses. The work of Claude (35) is cited in support, since he had demonstrated the presence of ribose-nucleoproteins in the particulate components of normal and cancerous cells of the chick and mouse and also suggested the etiologic significance of abnormal mitochondria in cancer. Further evidence along this line is found in other reports of Woods and Du Buy *et al.* (58, 59, 142, 146, 145), especially in the last cited paper which presents morphological and cyto-chemical evidence and which can be summarized as follows: Melanin occurs in the cytoplasmic granules of the melanoblasts of the Harding-Passey and Cloudman S91 mouse

melanomas and in the phagocytes of these tumors. The cytoplasmic granules (melanized and non-melanized) are the only structures which stain with Janus Green B (supposedly specific for mitochondria). No other structures resembling mitochondria are visible in the cells. On alkaline hydrolysis these granules apparently yield ribonucleic acid, as suggested by the ultra-violet absorption band at 2580 Å, and contain phosphorus and pentose. Centrifugally isolated cytoplasmic granules of amelanotic melanoma cells possess enzymic activities characteristic of the mitochondria of other cells, i.e., liver, etc., such as the cytochrome and succinic oxidase systems (cf. 14).

They conclude as follows (145): "Mitochondria are fundamental structures in both animal and plant cells, and in plants it is now well established that they derive from pre-existing mitochondria, possess a complex hereditary system (chondriogenes), and are capable of mutation. In mutant states plant mitochondria display abnormal enzymic activities. Self-duplication of these abnormal mitochondria results in the development of neoplasia. Plant mitochondria may be specifically modified by certain viruses that result in neoplasias very similar to those caused by mutant chondriogenes. The behavior of normal, mutant or virus-modified mitochondria may also be affected by specific nuclear genes".

And further (142): "Mitochondria, changed by a process of 'retrograde' evolution, could be considered as the underlying cause not only of the plastid-controlled variegational diseases in plants and ultimately of the viroses, but also, through a similar course in evolution, as the underlying causes of many cancers. As far as we are aware, the first experimental data to be presented pointing to such a fundamental relationship among these three types of diseases [virus diseases, variegational diseases and cancer] are given in our earlier papers".

A similar theory for the origin of neoplasms was advanced by Altenburg (1, 2), and if the mitochondria are substituted for his "viroids", the case is essentially the same as postulated by Woods and Du Buy. Haddow (72) also found the evidence of Woods and Du Buy acceptable, and Bald (6) presented further corroboration.

There is considerable presumptive evidence of a collateral kind, suggesting a causal relationship between mitochondria, morpho-

genesis and heredity. Brachet (17) isolated the cytoplasmic nucleoproteins in the microsomes and found these granules also to contain important respiratory enzymes, such as peroxidase, succinic-dehydrogenase and cytochrome oxidase. In addition, acid and alkaline phosphatases, amylase, dipeptidase, cathepsin, trypsin, arginase, adenylic acid deaminase and ribonuclease were located on the microsomes and in the supernatant fluid. Brachet notes a close resemblance between virus prolification and the spreading in the embryo of the normally inducing substances, and suggests that the multiplication of these ribonucleic acid granules in the egg might be of considerable importance in morphogenesis and also asks whether these granules might represent plasmagenes responsible for cytoplasmic differentiations. He also observed (18) that regions of highest morphogenetic activity were richest in ribonucleic acid and highest in rate of respiration. In this connection it is pertinent to recall that mitochondrial frequency is also highest in tissues of high metabolic rate. Brachet states that whether we call these granules "plasmagenes" is only a question of definition, since we do not know enough of their possible genetic significance. With increase in size they also become more complex. Brachet and Jeener (21) do not associate these granules, which, however, correspond to the microsomes of Claude, with mitochondria. Brachet (18) did find the enzyme amylase in an amoeba associated with mitochondria. Since Claude has found an association and chemical similarity, both as to structure and enzyme content, between microsomes and mitochondria, it is perhaps plausible to suggest a similar role for mitochondria. Since they are associated with enzymes and do contain ribonucleic acid, their frequency variations in different tissues, especially their abundance in meristematic tissues or other cells of high metabolism and their reduction in numbers concomitant with morphogenesis, may be explained by the following: Spiegelman (132) has shown that the removal of a substrate leads to the disappearance of the enzyme system it evoked; Bensley (12) observed the corresponding disappearance or reduction in number of mitochondria during periods of inanition and their reconstitution after feeding; and Brachet (17) observed a substantial decrease in the ribonucleic acid content in fasting animals.

Monné (102) has ascribed properties similar to those suggested

for mitochondria and microsomes, to structures called "chromidia" by him. These chromidia are not the chromidia of Goldschmidt, emanating from the nucleus, but are cytoplasmic, self-propagating fibrils, fundamental to the metabolism and ontogeny of the organism, according to Monné, and are probably the equivalent of the microsomes.

It would seem to the reviewer that much of the discrepancy between the cyto-chemical and morphological data of the investigators might be explicable in terms of technique. Since all used a maceration-ultracentrifuge technique, and since the technique is at least sometimes capricious and requires careful manipulation (see 54) for successful results, greater practice and perhaps improvement of the technique should produce more equivalent results. Brachet (20) has recently reported the isolation of five different intracellular fractions, all of which differ in chemical composition.

In connection with the enzymatic properties of mitochondria and their possible role in heredity, the papers of Spiegelman (132, 133), based upon his researches on yeasts, are of special interest. He found that the nucleoproteins are the controlling elements in enzyme synthesis and suggested that these phosphorus-containing proteins are the specific energy donors which make possible the reactions leading to protein and enzyme synthesis. Caspersson (30) found the granules in yeast cells rich in ribonucleic acid. Caspersson, Brachet and others agree that the nucleus contains all or nearly all the desoxyribose nucleic acid of the cell, whereas the ribosenucleic acid is found largely in the nucleolus and cytoplasm. Since Claude, Zollinger, Woods and Du Buy, *et al.*, have further localized the ribosenucleic acid in the mitochondria, their progenitors or derivatives, an at least tentative identification of mitochondria with the nucleoproteins of Spiegelman may be indicated. According to Spiegelman and others, all self-duplicating entities have been found to be linked with nucleic-acid-containing compounds; among such entities may be mentioned genes, plastogenes, viruses and the pneumococcus "transforming principle". Cellular enzyme formation is primarily instigated by the nuclear genes, whose main function is their indefinite retention for the cell of the potentiality for enzyme formation. However, the actual formation of an enzyme in the cytoplasm is mediated directly by a

cytoplasmic unit called a " plasmagene ", which possesses the capacity for self-duplication in the presence or absence of the corresponding gene. These plasmagenes are considered as gene replicas which enter the cytoplasm, presumably during mitosis in the absence of the nuclear membrane, and, like all self-duplicating entities, compete with each other for protein and energy. The outcome of such competitive interactions then determines the enzymatic constitution of the cytoplasm. Thus the genotype determines the phenotype by virtue of genic control over enzymic synthesis, and a mutant results in a modification of the cytoplasmic enzymatic constitution. Spiegelman has shown that the genic control over enzymatic adaptation is not unlimited, by placing a population of cells in contact with a substrate for which the cells possess no enzyme of genic origin for assimilation and observing that, after a lapse of time, the enzymes necessary to metabolize the added substrate appear. Such " adaptive " enzymes differ from the " constitutive " ones by disappearing upon removal of the substrate, whereas the latter are presumed to be synthesized by the cell at all times. " The plasmagene by its very nature must possess heterocatalytic potentialities—i.e., in addition to being autosynthetic, it must possess the capacity for catalyzing the synthesis of units (enzymes) other than itself " (132). A plasmagene, once formed, according to Spiegelman, can not, of course, exist indefinitely, particularly in a population of other such units actively competing for the material of which it is composed. In the absence of an homologous substrate it is broken down to inactive protein, losing its capacities for self-duplication and enzyme formation. Furthermore, " inherent in the definition of a self-duplicating entity is the concept that, should such a unit undergo a modification at any given moment, all subsequent replicas would bear this modification. Whether a particular character (enzyme) will be transmitted from one cell generation to another in a Mendelian fashion will thus depend on the relative rates of duplication of the controlling cytoplasmic units as compared with their rate of production from the genome. If the latter is quantitatively determining, Mendelian inheritance will be observed. If the former is determining, the Mendelian picture will be obscured to varying degrees, depending on the self-duplicating capacity of the plasmagenes. It is also clear how a substrate could so intensify cyto-

plasmic inheritance of a particular enzyme as to completely obscure the segregation of the corresponding gene". This theory, further developed by Spiegelman (see also 133), provides a point of view from which such diverse and apparently contradictory phenomena as classical Mendelian genetics, cytoplasmic inheritance, cellular differentiation and enzymatic adaptation may be analyzed, and "the basic problem of cancer involves explaining the appearance of a sudden heritable change in somatic cells, analogous in several ways to enzyme adaptation or cellular differentiation". In this connection one need not accept Spiegelman's conception of the plasmagene as of nuclear origin. The definition of the plasmagene is a loose one, and any self-duplicating cytoplasmatic particle might be so-called (cf. 49, p. 207 ff.)

Application of this experimental work of Spiegelman to a consideration of the functional significance of mitochondria seems obvious, and if one substitutes the term "mitochondria" for "plasmagene" in the foregoing, a definitive theory appears, which is adequately supported by both experimental and morphological evidence. Morphologically the theory is supported by the common observation of the greater abundance of mitochondria in tissues of high metabolism, such as meristems and undifferentiated embryonic cells, which would be relatively rich in substrate allowing full development of competitive enzyme systems localized on mitochondria, and the subsequent numerical reduction of mitochondria with tissue specialization concomitant with morphogenesis. The pleomorphism of mitochondria is also explicable in terms of the variety of enzyme systems localized on mitochondria, the varying rates of synthesis, dependent upon the amount and kind of substrate involved, and the fluctuations of heterocatalytic and autocatalytic reactions regulated by the kinds and amount of substrate present.

Identification of the plasmagenes of Spiegelman with mitochondria seems implied by his concluding remarks in further clarifying his definition of the plasmagene. "The plasmagene as defined in the present paper is a more or less complete gene-replica, which possesses to a varying extent the capacity to self-duplicate. It is not a special or unique cytoplasmic component in the sense that it is outside normal physiological processes. It is an integral part of the enzyme-synthesizing system and is the normal link by means

of which genes can effect control over protein formation in the cytoplasm. Whether or not plasmagenes are 'molecular' is not pertinent to their definition. It seems probable, however, that they would perform their synthesizing functions on the surfaces of relatively large particles (cytoplasmic granules) [*sic*] which could provide the necessary protein and energy-rich groups".

Further elaboration of this theory for the function of mitochondria in morphogenesis seems provided by Spiegelman (133) from his investigations on yeasts (see also 149 and 130). According to him, the functional and reactive capacities of a cell are determined by the enzymes it contains, and differentiation is paralleled by a progressive change in the quantitative distribution of the various enzymes, i.e., some are increased and some are suppressed; morphogenesis is thus a study of enzyme change. A summary of his conception of gene action is as follows:

(*a*) Genes produce at various rates more or less complete replicas of themselves, which enter the cytoplasm.

(*b*) These replicas (or plasmagenes) are nucleoprotein in nature and possess to varying degrees capacity for self-duplication.

(*c*) Their presence in the cytoplasm controls the types and amounts of proteins and enzymes synthesized.

(*d*) These plasmagenes, like all self-duplicating entities, compete with each other for proteins and energy, and the outcome determines the enzymatic constitution of the cytoplasm.

The plasmagene, by nature, must be not only autosynthetic but also heterocatalytic, i.e., it must be able to catalyse the synthesis of units (enzymes) other than itself. The plasmagenes can not exist indefinitely but disappear in the absence of an homologous substrate. Rapidly dividing cells thus tend to maintain their enzymatic patterns, and marked changes in enzyme content and, presumably, differentiation occur only when cell division slows down. It may be reiterated parenthetically here that mitochondria frequency parallels this enzymatic pattern. Morphogenesis is thus due to the competitive interactions of enzyme systems. Identification of mitochondria with the plasmagene is again suggested by Spiegelman's presumption that any cytoplasmic particles rich in nucleic acid should also contain a large number of enzymes.

These results seem to supplement the findings of Claude, Hogeboom, Schneider, Woods, Du Buy and others, who have isolated

the nucleic acid and enzymes essential to morphogenesis in the mitochondria of the cytoplasm of plant and animal cells. Thus the desideratum of Spiegelman as to what are these cytoplasmic self-duplicating and transmissible units most closely associated with the nucleoprotein fraction seems to be exclusively supplied by mitochondria, and these necessary particles or plasmagenes are now at least tentatively provided with "a local habitation and a name". Further work will, of course, be necessary to validate the association.

The plastogene theory of Imai (84) is a particulate application of this theory to plant plastids. The plasmagene theory of Sonneborn (128, 129, 130) and the genoid of L'Heriter (90), invoked to explain the non-Mendelian inheritance of CO_2-sensitivity in *Drosophila,* male sterility in plants and other cases of cytoplasmic inheritance, as well as "Dauermodifications" in general, may also have reference to mitochondria, but whether any single theory will explain the role of the cytoplasm in heredity, it is impossible to say at this time.

SUMMARY

The significant researches of the past decade on cytoplasmic inclusions have been characterized by new experimental morphological and cyto-chemical techniques which have shed new light upon the role of mitochondria in plant and animal cells. Although some of the experimental approaches were motivated by considerations of non-Mendelian heredity, viruses, enzymology and neoplasms, and the results are sometimes tangential in reference to mitochondria, there appears to be a sufficient correlation between these sometimes oblique approaches and those concerned with mitochondria per se to establish a working theory for the functional significance of mitochondria.

Techniques for the isolation of mitochondria from the cell by maceration and ultra-centrifugation, improved cytochemical and genetic techniques, the application of electron and phase microscopy and other morphological techniques to the problem, have rather clearly indicated the relation of mitochondria to metabolism, morphogenesis and heredity, and have further implicated them with viruses and neoplasms of plant and animal cells. Further work will be necessary to clarify these relationships, but with the

techniques now available the way seems clear for more definitive evidence.

The advances—but also the limitations—of the experimental techniques have emphasized the need for further researches on the more orthodox morpho-cytological techniques for the better fixation and staining of cellular inclusions, and they suggest the search for new chemicals for fixation rather than further modifications of extant formulae. This task may have been made easier by the cyto-chemical revelations made thus far of the properties of the protoplasmic constituents.

It can be stated that greater progress has been made in the past decade concerning the functional significance of mitochondria than in the preceding 40 years.

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APOGAMY, APOSPORY AND PARTHENOGENESIS IN THE PTERIDOPHYTES. II[1]

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INTRODUCTION

There can be no doubt that the plants most favorable for the study of apogamy and apospory are the homosporous leptosporangiate Filicales, in many of which these deviations in life cycles have been found to occur in nature and also under cultural conditions. They are not, however, at present fully understood. In a large number of ferns, apogamy is known to be a constant phenomenon, but apospory of this nature has been found in only a single fern, *Scolopendrium vulgare*. The discovery of its constant occurrence was made as a result of the studies of the inheritance of apospory in certain ferns by Andersson-Kottö (1931, 1932) and Andersson-Kottö and Gairdner (1936).

Both apogamy and apospory are sometimes described as abnormalities in the life cycle of a plant. It is commonly assumed that if one of these phenomena occurs in a life cycle, it must be followed by the other one. However, it is known that only apogamy occurs in some ferns, and that apogamy is the only way in which the sporophyte is reproduced in those cases. In some apogamous ferns apospory may occur, but it may also be found in non-apogamous ones.

It is suggested by the writer that both apospory and apogamy may be considered as normal phenomena in the life cycle, and are not necessarily related. For a brief discussion of the independence of these phases in a life cycle, the reader is referred to an earlier paper by the writer (1939).

Studies of apogamy and apospory in the Pteridophytes during the decade just past (1939–1949) have not been numerous. Hence this group of plants, so favorable for cytological study of these phenomena and others of a physiological nature, has interested comparatively few botanists. During the past ten years, however, a number of papers have appeared on various phases of apogamy and apospory as follows:

[1] Supplement to article in The Botanical Review **5**: 433–453. 1939.

1. The discovery of apogamy in several ferns.

Apogamy was discovered by the writer in three ferns: *Tectaria trifoliata* (1944*a*), a *Dryopteris* species (1944*b*), and in *Pellaea ovata* (Unpublished).

In *T. trifoliata* the gametophytes are usually variegated and are similar to those described in several ferns by Andersson (1923) and Andersson-Kottö (1930). The gametophytes produce antheridia in abundance, but no archegonia. Embryos are formed apogamously, usually on a projection produced from the sinus of the prothallium.

In an unidentified species of *Dryopteris* the writer (1944*b*) found that the embryos are formed apogamously, although the gametophytes produced in abundance both sex-organs. The gametophytes are more distinctly variegated than those of *T. trifoliata.*

Pellaea ovata was found to be constantly apogamous. The gametophytes of this fern are somewhat smaller than those of *T. trifoliata* and the *Dryopteris* species. In some of the numerous cultures of prothallia of the fern made by the writer over a period of 14 years, antheridia were always produced in abundance, but archegonia have never been observed on any of the gametophytes.

No detailed study of the cytology of apogamy of all these ferns was made by the writer. It has been found, however, that there is no change in the chromosome number in the life cycle of *Pellaea ovata.*

Apogamy was also discovered in *Angiopteris australis* by Stokey (1948).

2. The discovery of apospory in *Tectaria trifoliata* by Steil (1944*a*).

3. A study of the aposporous growths by the writer (1949) in *Pteridium aquilinum,* in which Farlow (1889) described apospory.

4. Induced apogamy in *Doodia caudata* by Duncan (1941).

5. Apogamy of the obligate type was also reported by Stokey (1942, 1948) in *Marattia sambucina* and in *Trichomanes auriculatum.*

6. The origin and development of the apogamous embryo by Duncan (1941, 1943).

7. The cytology of apogamy by Döpp (1939) and others.

Neither apogamy nor apospory has recently been discovered in any of the fern allies, and parthenogenesis has not been reported in any pteridophyte since 1939.

Apogamy has recently been reported in one of the cycads, all members of which have retained certain fern characters, especially in the foliage leaves and in some anatomical features. In one species of the group *Cycas Thouarsia,* Ducharte (1888), in germinating seeds, observed the formation of roots from the endosperm, but apparently failed to recognize the apogamous regenerative growths, since he was perhaps unaware of Farlow's (1874) discovery of apogamy in *Pteris cretica* var. *albo-lineata.*

Similar regenerative growths were described by LaRue (1948) who found that when the megagametophytes of *Zamia floridana* were removed from the ovules and cultured, they produced regenerative growths, including roots and leaves which resembled those formed by ordinary seedlings of the plant. LaRue found that the megagametophyte, when it had reached maturity or just after fertilization, seemed to possess greater regenerative tendency than at any other stage of its development.

Great care was taken to keep the cultures free from contamination. An ample supply of water seemed to be the important factor for producing the regenerative growths which were undoubtedly apogamous in nature.

The megagametophytes in the cultures increased, sometimes enormously in size, and produced apparently meristematic regions and protuberances and even, although rarely, roots and leaves. These vegetative organs were, however, usually formed independently and hence were not aligned. However, one plant was obtained in which the leaves and the roots seemed to be connected, but it unfortunately lived only about a year.

Excised portions of the gametophyte behaved in a somewhat similar manner, since they also produced apogamous growths.

Of frequent occurrence in the gametophyte of *Zamia,* observed by LaRue, was periderm, formed in successive layers, beginning near the outer surface. The periderm, which is an essential portion of the bark of the stem and root of gymnosperms and many angiosperms, is sporophytic tissue and hence is apogamous in origin in the megagametophyte. A similar periderm was reported by Merry (1936) to form in the endosperm of *Crinum asiaticum.* It was produced, however, only from the superficial cells which cannot be regarded as gametophytic. Therefore, the periderm in such an endosperm is not apogamous in origin.

LaRue also observed in some of the cultured gametophytes, tracheids, similar to those ordinarily produced in the prothallia of apogamous ferns. The cytology of the regenerative growths was not investigated, but LaRue suggests that they are haploid.

The discovery of apogamy in *Zamia* should lead to similar studies of the female gametophytes of other gymnosperms, including *Ginkgo biloba.*

ORIGIN AND DEVELOPMENT OF THE APOGAMOUS EMBRYO

Farlow (1874), who discovered apogamy in *Pteris cretica* var. *albo-lineata,* described in a general way the origin of the embryo. According to his description, it originates usually on the ventral side of the prothallium, a short distance back of the apical notch. From his somewhat detailed account it may be concluded that it originates from a number of cells of the gametophyte. Farlow, however, made no statement in regard to the origin of the apical cells of stem, root or leaf. He gives the order of development of these organs: the leaf first, then the root, and later the stem. A cursory study of the origin of these parts of the apogamous embryos has confirmed these observations. Farlow also discovered that an embryo apogamously produced always lacks a foot. Since there is no sharp line of demarkation between the cells of the apogamous embryo and those of the gametophyte, the foot seems unnecessary for development of the embryo.

Farlow observed that when the apogamous embryo was about to begin its development, tracheids usually made their appearance in the prothallium. These vessels could be readily distinguished in the gametophyte which later produced the embryo. They have been found in the prothallia of all ferns known to be apogamous.

For the origin of the apogamous embryo of two ferns, Farmer and Digby (1907) described a most unusual history. In *Lastrea pseudo-mas* var. *polydactyla* Wills and *L. pseudo-mas* var. *polydactyla* Dadds, the two apogamous ferns, they described the origin of the embryo from a single diploid cell produced by the fusions of two prothallial cells and their nuclei. The fusion of these cells, in the cushion of the gametophyte, was described as a "substitution" fertilization. Since the investigation by Farmer and Digby was mainly of a cytological nature, the stages in the development of the embryo were not followed.

After the publication of what seemed to be a remarkable account of the origin of an apogamous embryo of a fern, numerous studies of the embryony of apogamy have been made, but so far no one has confirmed the observations of Farmer and Digby. Döpp (1939) studied the origin of the embryo in these *Lastrea pseudo-mas* varieties and failed to find the fusions which had been described by these botanists. Döpp stated that the observations of Farmer and Digby were based on artifacts due to fixation.

Yamanouchi (1908) described the origin of the embryo in *Nephrodium* (*Dryopteris*) *molle,* which is a non-apogamous fern, under ordinary cultural conditions. He stated that when the prothallia were watered only from below and, hence, there was an insufficient amount of water for fertilization, the gametophytes produced the embryos apogamously. He also exposed the cultures to direct light. These two conditions were first found by Lang (1898) to be necessary for inducing apogamy in a fern in which embryos, under favorable conditions, are formed only sexually.

The apogamous embryo, presumably induced in *N.* (*D.*) *molle,* originated, according to Yamanouchi, from a single haploid cell on the ventral side of the prothallium a short distance back of the apical notch. The initial cell appears early in the development of the gametophyte, usually when the gametophyte becomes heart-shaped and the cushion begins its development. The cell could be readily distinguished by its larger size, larger nucleus and denser contents.

Duncan (1943) studied the origin and the development of the apogamous embryo in several ferns, including the two *Lastrea pseudo-mas polydactyla* varieties in which Farmer and Digby had described a "substitution" fertilization and hence the formation of a diploid cell, the initial cell of the embryo. However, in none of the ferns in which embryony was investigated by him could such fusions be observed. Neither could Duncan trace the origin of the embryo from a single haploid initial cell of the gametophyte as Yamanouchi (1908) had described in *Nephrodium* (*Dryopteris*) *molle,* in which apogamy was induced.

The origin and development of the apogamous embryo has been investigated in a number of ferns by the writer, who found the apparently binucleate condition in an early stage of the embryo due often to difficulty in staining the delicate walls of the young cells.

Occasionally it was also observed that some of the newly formed walls stained unevenly. Hence if the walls of the young cells are not well stained, a cursory study may lead to the conclusion that cell and nuclear fusions occur in the prothallium when an apogamous embryo begins its development.

According to Duncan, no evidence of the origin of the apogamous embryo can be observed until the prothallium back of the apical notch consists of four layers of cells. The upper two layers remain distinctly gametophytic, and hence are not involved in the formation of the embryo. From the two lower layers the whole embryo develops. The apical cell of the stem is produced from the fourth or lowermost layer. A segment of this apical cell functions as the apical cell of the leaf. In the third layer, from the upper surface of the prothallium, the apical cell of the root is differentiated. Since in an apogamous embryo, no study of the origin of the apical cell of the stem of the apogamous embryo had been made before, it was assumed that it originates independently and not from the apical cell of the leaf, as described by Duncan.

Although Duncan described a typical origin and development of the embryo in ferns in which there is the obligate type of apogamy, he observed that irregularities occasionally occur in some of the early stages of development.

Duncan (1941) also described the origin and development of regenerative apogamous growths, including the apogamous embryo in *Doodia caudata,* in the gametophytes of which he had induced apogamy. He found that the embryo induced apogamously in a fern is in most respects different in origin and development from that observed in the *Dryopteris* species in which apogamy is of constant occurrence. For a brief description of Duncan's observations on the embryo of the facultative type of apogamy, the reader is referred to page 97.

INDUCED APOGAMY

Lang (1898) was the first to make a study of "induced" apogamy in a number of ferns in which under ordinary conditions the embryo is produced only as a result of fertilization. Sometimes the conditions found by Lang to be necessary for inducing apogamy may exist in nature. He, however, studied these conditions in cultures of prothallia. The main factor for inducing apogamy was

found by him to be the amount of water supplied to the prothallia. When there was an insufficient supply of water in the culture so that fertilization was prevented, the embryos could not be produced sexually, but apogamous embryos were induced, sometimes in large numbers. When the cultures were maintained for a long period of time, the gametophytes formed various kinds of regenerative growths which seemed to be favored by direct light. The cells of the gametophyte under optimum light produce and store a larger amount of carbohydrate food, and at the same time they are stimulated to greater activity. Hence, according to Lang, meristematic regions are formed by the gametophyte. These regions of actively dividing cells were believed to be an important factor in producing the growths of the prothallia.

In the cultures of gametophytes of 17 species and varieties of ferns, Lang described three kinds of regenerative growths: archegonial projections, cylindrical processes, and the sporophytic buds. Unless the archegonial projections possess at least one sporophytic character, such as stomata or tracheids, they are not of apogamous nature. The cylindrical growths usually possess tracheids and hence are apogamous. The sporophytic buds are undoubtedly apogamous, although they may not always produce a complete embryo.

Induced or facultative apogamy has been described in homosporous ferns by numerous workers, including Leitgeb (1885), Stange (1887), Heim (1896), Lang (1898), Yamanouchi (1908), Heilbronn (1910), Schlumberger (1911), Nagaii (1914) and Wuist (1923).

Black (1909), Mottier (1915, 1927) and Steil (1918) failed to induce apogamy in the prothallia of certain ferns which were grown under the cultural conditions described by Lang and others.

The only recent investigation of induced apogamy was made by Duncan (1941) in *Doodia caudata*. The source of the spores for the cultures of the prothallia were produced by a plant obtained by W. H. Lang from the Kew Gardens and which was similar to five other plants in the experimental gardens of the University of Manchester where much of the work on induced apogamy of the fern was done by Duncan.

It was observed that the plant from the Kew Gardens differed from the other five in both color and texture of the leaves. It was

also found that the gametophytes of the fern differed from those of the other ferns in color, the number of hairs produced on the surface, and in their response to the culture conditions necessary for inducing apogamous growths.

Duncan confined his study of induced apogamy to the prothallia produced from the spores of the plant obtained from the Kew Gardens. When the prothallia produced from the spores of the other five plants were grown under conditions found necessary for inducing apogamy, the results were negative. However, under favorable cultural conditions the prothallia obtained from the spores of all six plants produced embryos sexually.

When Duncan flooded the prothallia of the different strain, sexually produced sporophytes were formed in abundance. These were removed when they appeared, and the cultures were kept for a prolonged period of time with no additional water supply, but only one of four remaining gametophytes produced an apogamous bud.

When the prothallia in some of the cultures were supplied with only a capillary source of water, only a few sporophytes were sexually produced. These were removed and the cultures were continued under favorable light conditions. Only, however, when the prothallia were kept in culture for many months, regenerative growths appeared. Some of these were gametophytic and others sporophytic, and in most respects similar to those described by Lang and others.

Duncan also studied the regenerative growths, including the apogamous embryo, the initial cells of which were found to be somewhat anomalous. Frequently the apical cell of the leaf appeared first. The apical initials of stem, root or leaf frequently failed to make their appearance. One characteristic of the development of the embryo of the facultative type of apogamy is its anomalous behavior. It is not difficult, therefore, to distinguish it from the embryo of the obligate type.

The studies of Duncan establish the validity of facultative apogamy and hence the work of Lang and others.

SOME RECENT CONTRIBUTIONS ON THE CYTOLOGY OF APOGAMY

A number of contributions have been made on the cytology of apogamy since the writer (1919) reviewed the studies of the

nuclear and cell phenomena which had been described in a number of ferns known to be constantly apogamous. Since it has not been observed in any fern in which apospory occurs, that there is no change in the chromosome number in the life cycle, it is obvious why no recent study of the cytology of this deviation in the life cycle has been made.

The first study of the cytology of apogamy in ferns was made by Farmer and Digby (1907) in *Lastrea* (*Dryopteris*) *pseudo-mas* var. *polydactyla* Wills and *L.* (*D.*) *pseudo-mas* var. *polydactyla* Dadds, in the gametophytes of which they described cell and nuclear fusions. The discovery of these fusions, or "substitution fertilization", was generally accepted by botanists and considered to be one of the greatest in the field of cytology. Undoubtedly its universal acceptance was facilitated by the classic paper of Strasburger (1894) on the "periodic reduction of the chromosome number in the life cycle of living organisms". Since it was found as a result of studies on plants possessing an alternation of generations that there is also an alternation of the chromosome number, it seemed obvious that the fusions described by Farmer and Digby confirmed the principle which had been apparently established by one of the greatest cytologists.

Döpp (1932, 1933, 1938, 1939), who had confirmed the observations of incomplete divisions in the sporangia of *Nephrodium* (*Dryopteris*) *hirtipes* described by Steil (1919), studied the origin of the apogamous embryo in the ferns in which Farmer and Digby (1907) had described the "substitution fertilization", but could not observe the fusions in the gametophyte before embryony.

Duncan (1943) made a careful study of the origin of the embryo in the *Lastrea* (*Dryopteris*) *pseudo-mas polydactyla* varieties and some other apogamous *Dryopteris* ferns, but observed no bi-nucleate cells and no fusions. In the apogamous ferns investigated by Duncan, the origin of the embryo could not be traced to a single haploid cell, as had been described by Yamanouchi (1908), but to a number of cells of the gametophyte, as has already been described.

Although apogamy was discovered in 1874 by Farlow in *Pteris cretica* var. *albo-lineata*, the cytology of the fern remained unknown until Heun (1939) studied the nuclear history in the life cycle of the fern. He found no fusions in the gametophyte preceding formation of the embryo sporophyte and no fusions of the

sporogenous cells in the sporangium, as had been described by Allen (1911) in *Aspidium* (*Cyrtomium*) *falcatum*. Hence Heun found that there is no doubling of the chromosome number and therefore no haplosis in the life cycle. He also observed that there was a high percentage of abortion of the sporogenous cells in the fern. No explanation was offered for the abnormal behavior of these cells in the sporangium which in some other apogamous ferns produce almost invariably normal spores.

Sr. M. Thomasine (Patterson) (1942) studied the cytology of apogamy in *Polystichum tussimense* and observed incomplete divisions similar to those described by Steil (1919) and Döpp (1932, 1938, 1939). The incomplete divisions in this fern usually occur before metaphase. They are followed by the formation of "restitution nuclei" which after haplosis produce normal spores. Only a small amount of abortion was observed in the sporogenous cells.

SOME RECENT CONTRIBUTIONS TO APOSPORY

In some cultures of the prothallia of *Tectaria trifoliata* the writer (1944*a*) observed that gametophytes originated from the first leaf of the apogamously produced sporophyte in three ways.

From one or more of the marginal cells of the leaf blade, prothallia were produced, frequently in large numbers. These were similar in appearance to those which were formed by germination of the spores. It was observed that there was always a sharp line of demarcation between the aposporously formed gametophyte and the sporophyte.

It was also noted that frequently in the first formed leaf of the sporophyte one or more of the gametophyte cells embodied in the leaf in early embryony produced the aposporous gametophytes, as had been described by the writer (1921) for *Polypodium irioides*.

In the same cultures there were also produced numerous gametophytes from the hairs borne by the petiole of the first leaf. Although numerous hairs were borne by the lamina, they never formed gametophytes. The terminal cell of the hair, which contained few chloroplasts, was often almost colorless and was not concerned in the formation of a gametophyte. The first readily observed change in the cells of the hair, before the aposporous gametophytes appeared, was the multiplication of the number of their chloroplasts. The gametophyte was sometimes produced from

a single cell, but frequently a number of cells were involved in the formation of a gametophyte.

Duncan (1941) also found that in some cultures of the prothallia the leaves of the young sporophytes sometimes produced aposporous growths.

Reference has already been made that Farlow (1889), who reported apospory in *Pteris aquilinum,* described the vegetative growths from the margins of the fronds of the fern as gametophytic and hence aposporous. The writer (1949), however, investigated the regenerative growths occasionally observed in *Pteridium aquilinum* var. *latiusculum,* found commonly in Wisconsin and undoubtedly like those of *P. aquilinum* in which Farlow reported the occurrence of apospory and concluded that the regenerative growths in the fern, although the cells are thalloid, are intermediate in character and hence not aposporously produced. Tracheids were observed frequently to extend outward into the growths. Stomata, however, were never found on any of the numerous growths which were removed and carefully examined with a microscope. The criteria for determining whether the growths formed sometimes so abundantly by the fronds of *P. aquilinum* were gametophytic are the following: the form of the gametophyte, usually heart-shaped in the homosporous leptosporangiate ferns; the form of the cell, usually more regular, as in the prothallium, sex organs, antheridia or archegonia or both; or the presence of rhizoids, a distinctly gametophytic character. Since the growths possess none of these characters, they are not gametophytic and hence not aposporous.

DISCUSSION AND CONCLUSIONS

During the recent decade (1939–1949) the contributions to apogamy and apospory have not been numerous, but they have nevertheless led to a solution of some of the problems concerning these deviations in life cycles, especially of homosporous leptosporangiate ferns.

Among the recent studies of great interest made since 1939 are those of Duncan (1941, 1943) on the origin and development of the embryo in a number of ferns with the obligate type of apogamy and also in a single fern with the facultative type. The studies by Duncan of induced apogamy are also of much interest, since they confirm the work of Lang (1898) and undoubtedly establish the

validity of facultative apogamy. The observations of Döpp (1939) and others on the cytology of apogamy are also of great interest and importance. During the decade apogamy and apospory have been reported in only a few ferns.

Duncan (1943) has shown, as a result of his studies of embryony in *Lastrea* (*Dryopteris*) *pseudo-mas* var. *polydactyla* Wills, *L.* (*D.*) *pseudo-mas* var. *polydactyla* Dadds, some other ferns with the obligate type of apogamy and in *Doodia caudata* in which he had induced apogamy, that the embryo cannot be traced to a single diploid cell, as had been described by Farmer and Digby (1907), or to a haploid cell, as had been described by Yamanouchi (1908) in *Nephrodium* (*Dryopteris*) *molle,* in which he had apparently induced apogamy.

Döpp first studied the cytology of the ferns in which Farmer and Digby (1907) had described nuclear and cell fusions in the prothallium a short distance back of the apical notch as a necessary step for the development of the apogamous embryo. Döpp (1939), however, observed no binucleate cells and no fusions, as had been described by these botanists. Duncan (1943), as a result of studies on the embryony of these ferns, could not confirm the observations of Farmer and Digby. The writer (unpublished) also studied the origin of the embryo in a number of apogamous ferns, including *Lastrea* (*Dryopteris*) *pseudo-mas* var. *polydactyla* Wills[2], but never observed binucleated cells or cell fusions in the prothallium. The observations of the writer on the origin of the embryo in apogamous ferns has also led to the conclusion that it does not originate from a single haploid or diploid cell.

Although Duncan (1941) established the validity of facultative apogamy, the conditions which are considered to be necessary for inducing development of an apogamous embryo are still somewhat in doubt. As has already been stated, some have failed to obtain apogamous embryos in certain non-apogamous ferns when the gametophytes for long periods were supplied only with capillary water and exposed to favorable light. It is suggested that there may be an additional factor of a genetic nature necessary for inducing apogamy in a fern. It is also suggested that the strain of *Doodia caudata,* in the prothallia of which Duncan induced apog-

[2] Spores for the cultures of the prothallia were obtained from Kew Gardens in 1933.

amy, differed genetically from the other five plants, all of which were also considered by Ballard as *Doodia caudata*. Since the gametophytes obtained from the spores of the plant also differed in some respects from those obtained from the spores of the other plants, their hereditary constitution of the gametophytes was not the same.

Undoubtedly some studies of facultative apogamy, including those of Yamanouchi (1908), have led to confusion. Duncan states that the embryo development which Yamanouchi (1908) described in *Nephrodium* (*Dryopteris*) *molle* does not conform to that of the facultative type, and hence is convinced that the embryos in his cultures were not induced by cultural conditions. Also, since the gametophytes of the fern produced the embryos early in the cultures, they were not, according to Duncan, of the obligate type. Perhaps the spores sown by Yamanouchi for his cultures of prothallia were not those of *Nephrodium* (*Dryopteris*) *molle* but those of some other fern in which apogamy constantly occurs.

Studies on the cytology of apogamy have shown so far that in some ferns, including *Pteris cretica* var. *albo-lineata*, there is no change in the chromosome number, as described by Heun (1939) and others in the life cycle, but that there are others in which the chromosome number is doubled in the sporangium as a result of incomplete mitosis, as described by Steil (1919), Döpp (1932, 1933, 1938, 1939) and Patterson (1942). It seems, therefore, from the studies so far made that there is no cell or nuclear fusion in an apogamous fern.

Further studies should be made of the conditions necessary for inducing the facultative type of apogamy, and also of the factors for obtaining aposporous regenerative growths. Since only one study has been made of apogamy with reference to its occurrence in some of the genera of the homosporous ferns, additional studies would no doubt be of interest. Perhaps the most interesting study would be on the cause of incomplete mitosis which occurs in the sporangia of some apogamous ferns. An investigation of this nature has already been made by the writer (1950).

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AMITOSIS. II [1]

J. McA. KATER [2]

Ten years ago the writer prepared a review of the above subject (Kater, 1940). It can safely be said that the intervening decade has brought forth no significant contributions in this field. The 1940 review was concluded with the following summary:

SUMMARY AND OUTLOOK FOR CLARIFICATION OF THE AMITOSIS PROBLEM

1. In some instances amitosis is unquestionably a non-reproductive process, probably necessitated by the specific functions of the tissues involved.

2. Amitosis is clearly reproductive only in the non-genetic macro-nucleus of the ciliate.

3. The so-called simple division of the normal protozoan nucleus is not amitotic.

4. That pathological amitosis of Protozoa is ever followed by cytodieresis is very questionable. Definite solution of the problem can be expected because all that is required is for those finding such nuclear divisions to watch similar specimens and see if they divide.

5. Division in the yeast ascus is not amitosis.

6. Division of the budding yeast cell is more than likely not amitosis. Definite word on the question should be forthcoming. Skillful application of the sometimes deceptive nucleal reaction should be very helpful.

7. The status of the amitosis question in highly specialized tissues of higher organisms has not been greatly altered in the past two decades. Opinion is still divided into three groups: (a) those maintaining that all amitosis is physiological and non-reproductive; (b) those believing definitely that it is reproductive; and (c) those who leave the matter as still open.

[1] Supplement to article in The Botanical Review **6**: 164–180. 1940.

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8. If the observations of MacMahon and Stough where wholly accredited and their interpretations accepted, the reproductive character of amitosis would be established.

9. The statistical approach followed by Stough in trying to determine if mitosis were adequate to account for growth may, if followed sufficiently far, contribute to a solution of the amitosis problem.

10. Search for cytodieresis (MacMahon), the critical point in the controversy, could be regarded more hopefully if approached by use of techniques of fixation designed to accentuate cell boundaries, rather than conventional histological methods. There is no reason why we can not hope for a solution on this basis.

11. Without a great change of procedure it seems unlikely that tissue culture will afford a good approach to the reproductive possibility of amitosis.

12. The tapetum, ordinarily classed as a case of physiological and occasionally reproductive amitosis has been definitely placed in the mitosis category.

13. The division of Cyanophyceae and bacteria can not yet be profitably compared to that of other forms.

14. Though the data do not permit a definite conclusion, this reviewer doubts that amitosis will prove to be a method of cell reproduction.

There was one paper published prior to 1940, Richards (1938), which was not included previously because its approach, although refreshingly new, did not appear to be valid. Richards determined the influence of colchicine on yeast growth, and because it did not retard growth he concluded that he had evidence for the amitotic reproduction of yeast. This reviewer does not concur in that conclusion, but because of the newness of the approach and the thought behind it, it has since been concluded that the colchicine technique should be considered in connection with amitosis.

The past decade has brought forth a few papers on the physiological type of amitosis (Jones, 1945; Worley, 1942). Likewise some research has continued on the subject of pseudo-amitosis produced by toxic agents or culture conditions. Conrad (1941), Garriques (1940) and Sorokin and Sommer (1940) will serve

as examples of contributions in this field. The general state of our knowledge has not, however, been materially altered.

As was indicated in the above quoted summary, as of 1940, the yeasts were the most difficult group of organisms to harmonize with the generalizations that were justified for other groups. The 1940–50 research papers on amitosis include more on yeasts than on any other material. Naidu and Bakshi (1946), Srinath (1946) and Subramaniam and Ranganathau (1945*a*, 1945*b*) all favor interpreting the kinetic figure as mitotic. Unfortunately their descriptions and figures are not so detailed as could be desired. The work of this group of investigators is, however, very significant because it indicates that some of the disagreements concerning mitosis vs. amitosis in yeast may be due to errors in current interpretations of the analogies of the interkinetic yeast cell and the cell of higher plants.

One paper has appeared which favors interpreting amitosis as a reproductive phenomenon in yeasts (Beams, 1940). This paper also included a rather shocking quotation from Conklin's 1917 review, so given as to indicate that amitosis was a reproductive phenomenon when actually it is universally recognized that Conklin, at the time his review was written, was one of the most uncompromising advocates of the physiological interpretation of amitosis.

Nagel (1946) has attacked the yeast problem in a commendably thorough manner. With carefully controlled culture conditions, she has examined earlier interpretations of the interkinetic cell and has broken with the tendency to analogize yeast cell organelles with those of higher plant and animal cells Likewise she breaks with efforts to analogize the kinetic figures. If time substantiates this view, we will have an explanation for past confusion on yeasts. Although this reviewer doubts her conclusions, her work has crystallized a vague impression that research on the cytology of yeast has not been starting at the proper point, and has been assuming analogy rather than studying it.

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THE DEVELOPMENT OF SPHAGNUM BOGS IN NORTH AMERICA. II[1]

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A considerable amount of new and significant information in regard to the development of sphagnum bogs on the North American continent has been made available since publication of the earlier paper (Rigg, 1940). Geographically our knowledge has been extended by field work in both northern and southern regions. This field work emphasizes the wide-spread occurrence of some features of bog development, many of which were already known in some localities. There is also some new information on bog development in a few parts of the United States in which the general course of the development of sphagnum bogs was already known. A few papers published before 1940 also merit attention. The use of layers of volcanic ash ("volcanic glass") in bogs as a means of dating approximately and relatively the age of bogs has made notable advance during the last ten years.

During the course of field work in 1949 sponsored by the Division of Mines and Geology in Washington State Department of Conservation and Development the writer had opportunity for more extensive and detailed field work on the bogs of that State than he previously had. The objective of this project is to obtain data on the peat resources of the State and publish them in a forthcoming bulletin. The writer is indebted to Mr. Sheldon L. Glover, Supervisor of the Division, for permission to use data from this field work.

It has not been possible to quote from all papers that have appeared since 1940 which give information on stages of sphagnum bog development in North America or to quote from all of the letters which have been so kindly written by botanists in reply to inquiries by the writer. It is believed, however, that sufficient

[1] Supplement to article in The Botanical Review **6**: 666–693. 1940. This paper, like the original one, deals with the development of the bogs themselves and only incidentally with pollen analysis as evidence of the development of vegetation in the regions surrounding the bogs.

material has been used to give a general idea of the new knowledge in this field that has been made available during the last ten years.

NORTHERN BOGS

Southern and South Central Canada

Leverin (1946) worked on the peat moss (*Sphagnum*) deposits in all of the Canadian provinces that border the United States and also in Nova Scotia and Prince Edward Island during the years 1939 to 1943, inclusive. He investigated sphagnum from the standpoint of its commercial uses but he incidentally contributes a large amount of ecological information. He describes and discusses 136 bogs or groups of bogs, practically all of which have a layer of sphagnum varying in depth from two or three to as much as 30 feet. Most of these have living *Sphagnum* at the surface. In area they vary from two or three acres to 30,000 acres or more.

The number of bogs or groups of bogs described in each province varies from nine to 32 except in Alberta where he visited only a few. However, a number of bogs in Alberta have been described and discussed by Hansen (1949, 1949*a*).

Many of the bogs described by Leverin consist of practically pure sphagnum with no layers of peat formed by other plants. In many of these bogs the sphagnum peat rests on clay. On a number of them there is now a growth of trees and bog shrubs. The number of bogs in which the *Sphagnum* succession began on clay and continued to be the dominant up to the stage where the invasion of shrubs and trees began is worthy of note. Brief notes on a few bogs selected from the various provinces will characterize their present stages and give some idea of their earlier stages.

Nova Scotia. Glasgow Head Bog (50 acres) near Canso in Guysborough County is dry and "entirely free from wooded vegetation". The peat is ten to 15 feet deep and consists of sphagnum with some *Eriophorum*. Heath Bog in Yarmouth County consists of a chain of bogs running south from Sand Point for seven miles. The main part (Great Heath) is flat and shallow. The deposit consists of sphagnum peat. The surface growth is

Sphagnum, Eriophorum and *Hypnum,* intermixed with bog plants, *Carex* and other marsh sedges.

PRINCE EDWARD ISLAND. Black Banks Bog in Prince County comprises two dry, somewhat dome-shaped bogs, one 500 acres, the other 200 acres. Both extend to the shore, where sea erosion has exposed about ten feet of solid peat moss on a clay bed. Both have large open areas, but one is wooded toward the edges.

NEW BRUNSWICK. Gades Bog (1000 acres) south of Canaan Station in Westmorland County has "depths of 1 to 4 feet of humified peat with only a slight cover of *Sphagnum* moss". It was flooded at the time that it was visited. Large sphagnum bogs are numerous in New Brunswick, and some of them are deep. Eel River Bog in Kent and Northumberland Counties has an area of 15,500 acres of peat moss five feet deep. St. Stephen Bog in Charlotte County has an area of about 150 acres, 70 acres of which has peat moss varying from 12 to 30 feet in depth.

QUEBEC. Les Escoumains Bog in Saguenay County (area between two and three square miles) is of the high-moor type and has a fairly uniform depth of 11 to 18 feet of peat moss. "The (surface) growth is chiefly sphagnum moss and there is some rosemary, labrador tea, an abundance of blueberry bush, occasional small spruce islands, and scattered clumps of spruce trees". There are many small and a few large lakes which in some parts of the bog lie close together. The surface, when the bog was visited, was generally dry except in depressions near some of the lakes.

ONTARIO. Polar Bear Bog in Patullo and Nelles Townships is in the Rainy River district not far from the northern boundary of Minnesota, U.S.A. It covers the larger part of four sections, but the area bearing peat moss has not been determined. It ranges in depth from four to 12 feet. The upper four feet is sphagnum moss. Below that depth the material is more or less humified. "The surface growth is mainly sphagnum moss that is intermixed with *Hypnum* moss and sedges, labrador tea, bog rosemary and aquatic plants".

MANITOBA. The bog (area 1000 acres) east of the Winnipeg River near Lac du Bonnet village is very wet. The stratum of

peat moss is four to five feet deep, and underlying this is "peat of higher humification" to a depth of two or three feet. It has a very heavy growth of tamarack and spruce. The surface growth is sphagnum moss with some *Hypnum* moss and sedges. The bog west of the river is about six miles long and three miles wide. It was dry when visited. The depth of the peat moss stratum is four to five feet. "It has a heavy cover of live sphagnum moss with virtually no other growth of low plants. There are occasional fair-sized clear spaces".

SASKATCHEWAN. The bog south of Mistatim is ten to 12 miles long and six miles wide. It is soft and wet. The peat moss stratum is four and one-half to five feet deep and rests on well humified peat that is "packed so solidly that it could not be penetrated with the sampler drill". It is heavily wooded with small spruce, tamarack and birch. The surface growth is chiefly sphagnum moss with *Hypnum* and sedge in the open spaces.

ALBERTA. Leverin describes only one bog in Alberta. It is five miles west of Edmonton. So much of the peat moss has been removed from this bog over a period of years that the supply is practically depleted.

Hansen (1949, 1949*a*) has, however, in his papers on fossil pollen described 11 bogs, all within 125 miles of Edmonton. The bog in the poplar climax area near Lacombe has developed in a slough with a mat of sedge and *Sphagnum* and other herbaceous plants scattered throughout. The principal woody species are bog birch (*Betula glandulosa*), dogwood (*Cornus stolonifera*), black spruce (*Picea mariana*) and a few species of willow. The depth of the sediments in the area of sampling is three meters. (9.8 ft.), including almost a meter (3.3 ft.) of limnic peat which indicates a prolonged open water stage. Under this limnic peat is blue clay.

Four other bogs described by Hansen lie on the Keewatin drift and are "in the mature stage with climax forest trees on their surfaces". They vary in depth from one meter (3.3 ft.) to seven meters (22.9 ft.), but the relative depths "provide no criteria with respect to their relative ages". The deeper layers of volcanic ash in these bogs may have come from Glacier Peak in north central Washington or possibly from the Coast Range

in British Columbia. The ash near the surface of the bogs probably came from the eruption of Mt. Katmai in Alaska in 1912.

Hansen says: "In general the term muskeg has been applied to sites of peat accumulation in Canada and the Arctic, particularly in the tundra and boreal forest regions. Here the peat accumulation has occurred largely in shallow basins or on almost flat terrain by the growth of sphagnum moss and ericaceous shrubs". He says: "Even in muskegs that were formed in well-defined basins the stratum of limnic peat is thin and sedge peat is largely absent. The limited accumulation of limnic peat may be due in part to the oligotrophic character of these northern lakes, which in conjunction with the short cool summers, inhibits the growth of plankton and other organisms that contribute to the limnic peat".

Hansen distinguishes in general between the blue clay formed at the bottom of deeper muskegs (or bogs) and that formed at the bottom of the shallower ones. In the deeper ones the blue clay was washed in by water (allocthonous origin), while in the shallower ones the blue clay was formed in situ by gleization of the soil after an accumulation of peat had kept it water-logged for a long time (autocthonous origin). The absence or scarcity of pollen in the latter and its relative abundance in the former support this view.

BRITISH COLUMBIA. The bogs in this province discussed by Leverin are in three regions—The Fraser River Valley, Graham Island, and the Okanogan Valley. Bogs of the Fraser River Valley had previously been discussed by Anrep (1915), Oswald (1933) and Rigg and Richardson (1938).

Graham Island is the northernmost island of the Queen Charlotte group. There is a series of small bogs on the rolling land near Port Clement on the eastern side of the island. The bogs when visited were rather dry, and pools of water were found only in occasional places. The bogs are drained by slow creeks which flow into rivers. One of these bogs near Woodpile Creek has an area of about 20 acres and is surrounded by brush country with trunks of dead fallen and upright trees up to eight inches in diameter. The surface growth on the bog is sphagnum moss, bog rosemary, labrador tea, crowberry and sedges. The bog is

slightly dome-shaped at the summit and there the sphagnum moss is the thickest (18 inches). Many woody roots are present in the lower portion of this peat.

In the Okanogan Valley of British Columbia, Leverin investigated bogs near Penticton and Kelowna. His discussion of them is brief and does not disclose any important new information in regard to the development of sphagnum bogs.

District of Mackenzie

Raup (1947) found muskegs on the low ground at both ends of Brintnell Lake and on various local areas of small size on the mountain slopes and lake shores. This lake (3½ miles long, elevation 2600 feet, latitude 62°5′ N., longitude 127°31′ W.) is in the southwestern part of the District of Mackenzie, Northwest Territories. His general account of the structure and flora of these muskegs lists *Picea americana, Larix laricina* var. *alaskana, Ledum groenlandicum* and *Sphagnum* sp. as primary species. The secondary species are of the following genera: *Equisetum, Selaginella, Arctagrostis, Calamagrostis, Poa, Eriophorum, Carex, Juncus, Tofieldia, Corallorrhiza, Cypripedium, Habenaria, Listera, Salix, Alnus, Betula, Arenaria, Anemone, Ranunculus, Cardamine, Rorippa, Chrysosplenium, Parnassia, Ribes, Geum, Potentilla, Rubus, Hedysarum, Oxytropis, Empetrum, Epilobium, Andromeda, Arctostaphylos, Cassiope, Vaccinium, Gentiana, Pedicularis, Boshniakia, Pinguicula, Valeriana, Aster, Erigeron, Antennaria, Senecio,* and *Taraxacum.* The bulk of this muskeg flora is typical of this habitat throughout most of the Northwest. No raised bogs were seen, but on gentle well-watered slopes the muskegs have a tendency, as in so many other parts of the North, to spread upward. Some modifications of water levels and muskeg communities caused by beaver dams were seen along a sluggish stream.

Alaska

Dachnowski-Stokes (1941) made a field investigation of the peat resources of Alaska and included in his report a comprehensive account of the general course of development of each of the 43 bogs (muskegs) that he studied. He found slope muskegs, raised muskegs, and flat or valley muskegs.

Slope muskegs are common on gently undulating slopes in the exposed coastal regions bordering the Pacific Ocean and certain portions of Prince William Sound, and are especially prominent on Mitkof Island. *Sphagnum* (several species) forms hummocks which rarely dominate any considerable area.

Raised (dome-shaped) muskegs are well represented at Juneau and between Seward and Hope, and also in certain localities in the interior. The sphagnum moss peat in them frequently reaches several feet in thickness. Hummocks of *Sphagnum* are colonized by *Empetrum nigrum, Vaccinium Vitis-Idaea* var. *minus,* cranberry and sundew, while *Rynchospora* spp. and cotton grass occupy the intervening hollows. Raised muskegs develop in less extremely wet climatic conditions than those necessary for slope muskegs.

Representative flat (sometimes saucer-shaped) muskegs are found in the Copper River region and in the valleys of the Anchorage district; also in the interior. The normal succession in these muskegs is from aquatic peat-forming communities to sphagnum mosses and their associates. A few selected muskegs will illustrate these types.

1. Ketchikan muskeg (two acres) in southeastern Alaska occupies a trough-like depression scoured out of the rock by ice at an elevation of about 50 feet above sea level. This is a raised muskeg consisting, from top to bottom, of two and one-half feet of sphagnum peat (pH 4.0); one foot or less of woody peat with stumps and roots of conifers, roots and leaves of heaths and other materials; and two and one-half feet of sedge peat. Under this is six inches of sand with decomposed matter (pH 5.8). On the drier and firmer portions of the hummocks occur *Sphagnum fuscum*[2], *S. rubrum* and *S. squarrosum* which are the primary peat formers of the area. *S. cuspidatum* occurs in the wetter habitats. The usual bog herbs and shrubs of the region are present and there is a scattered growth of dwarfed conifers.

2. Point Higgins muskeg in the Ketchikan district is flat and lies between rock outcrops. The layers of peat are: 0 to ten inches, sphagnum with roots of woody plants; ten to 36 inches,

[2] All species and varieties of *Sphagnum* mentioned in the present paper are as either recognized or given as synonyms by Andrews (1913) except *S. imbricatum cristatum* Warnst. listed by Mohr (1901) and *S. rubrum* listed without author by Dachnowski-Stokes (1941).

sedge peat with some sphagnum and some roots of woody plants; three to seven feet, water and slime; seven to eight feet, sedimentary peat; eight to 10.5 feet, sedge peat; 10.5 to 12 feet, sand with remains of woody plants, grasses and sedges. The ground cover of living *Sphagnum* is not continuous. The bog is timbered with slow-growing conifers among which bog shrubs grow.

3. Petersburg muskeg is on Mitkof Island in southeastern Alaska. Sloping muskegs, relatively flat in some areas and interrupted in places by timbered areas, extend for several miles inland from the town of Petersburg which is on the coast and is built on muskeg. An area of five acres near the town was bored in several places. A general statement of the layers of peat in this area is: 0 to 16 inches, sphagnum peat varying in thickness and not continuous; 16 to 50 inches, sedge peat extending to the surface where there is no sphagnum peat; 50 to 56 inches, wood peat, stumps and roots; 56 to 96 inches, sedge peat; 96 to 102 inches, wood peat and stumps; 102 to 120 inches, sedge peat. Below this is sandy and clayey gravel. The bottom topography is sloping. In all probability peat formation has been continuous here since the retreat of the local glaciers.

4. Montana Creek muskeg, 16 miles from Juneau in southeastern Alaska, is partly flat and partly raised. It was originally a sedge marsh which later supported some timber but was soon replaced by *Sphagnum*. The layers of peat are: 0 to nine feet, sphagnum moss with methane gas at the bottom; nine to 17 feet, sphagnum peat with some *Hypnum* peat, woody fragments and remains of sedges toward the bottom; 17 to 20 feet, sedge peat. *Sphagnum* mosses are the really effective peat formers in this muskeg. The species present are *S. acutifolium, S. fuscum, S. rubrum, S. medium, S. papillosum* and *S. cuspidatum*. The dome-shaped portion has some pools and secondary lakes.

5. Gravina Point muskeg is near Port Gravina in Prince William Sound in south central Alaska. It is a sloping muskeg with a thin layer of sphagnum and sedge peat, under which is one and one-half to three feet of sedge peat merging into underlying sandy gravel. *Sphagnum* at the surface is growing rapidly and has formed hummocks. This muskeg has a growth of bog herbs and shrubs with scattered stunted spruce and hemlock.

6. Spenard muskeg about five miles from Anchorage in south central Alaska is concave in form and contains a shallow lake. Quaking mats of *Sphagnum* surround the open water of the lake. In the central portion of the muskeg active hummock builders (*Sphagnum medium, S. papillosum* and *S. fuscum*) cover the mounds, and *S. cuspidatum* and *S. cymbifolium* dominate the wet hollows. Aquatic plants grow in the margin of the lake. Near the margin of the open water the strata of peat are: 0 to eight inches, sphagnum moss peat with roots and rhizomes of growing sedges; eight to 60 inches, sedge peat with a thin layer of volcanic ash between three and four feet below the surface; 60 to 130 inches, *Hypnum* peat with remains of herbaceous aquatic vegetation; 130 to 140 inches, sedimentary peat merging at the bottom into sand.

7. Fairbanks muskeg in interior Alaska lies between the University Farm of the University of Alaska and the town of Fairbanks. It has an area of over 400 acres and lies in a basin-like depression. The central portion is dominated by actively growing *Sphagnum medium, S. fuscum* and *S. rubrum* which form hummocks. A profile at one point shows: 0 to three and one-half feet, sphagnum peat; three and one-half to four feet, woody sedge peat; four to six feet, moss and sedge peat with some woody material and some remains of mosses of the hypnum family. At six feet and below the material is frozen, even in summer.

Canadian Eastern Arctic

Polunin (1948) made field studies, beginning in 1931, of vegetation and ecology, mainly near Hudson's Bay posts or other points of call during Canadian Eastern Arctic expeditions. The region studied extends from 60° N. to 83° N. latitude. The northernmost subdivision studied was Ellesmere Island, and the southernmost were northernmost Quebec, northernmost Labrador and the west coast of Hudson Bay. The places visited are all within five miles of the coast and all were selected on account of their accessibility. The vast areas between these localities remain uninvestigated. Polunin gives accounts of the various plant communities in the places visited.

The present writer makes the following general statements

from reading these accounts. No *Sphagnum* is reported on Ellesmere Island. The northernmost report of *Sphagnum* is at Dundas Harbor (74°35′ N.) on Devon Island. *Sphagnum* is not dominant in any of the communities investigated and is never a pioneer alone. It is merely one of several mosses forming swards or mats or, in some places, tussocks. These mosses have not formed peat of any consequence. *Sphagnum* plays a progressively more important role in plant communities in which it occurs from North to South. Only in the extreme southern portion of the region, however, does it play an important role in the development of local plant communities, and even in these other mosses play important roles along with it. A brief statement of the ecological situations in which *Sphagnum* occurs at a few selected representative places follows.

1. Dundas Harbor (74°35′ N., 82°10′ W.) is on the south shore of Devon Island. On the moist but well-drained slopes in the lowlands the blueberry heaths on humous soil overlying a gneissic substratum are dominated by *Vaccinium uliginosum* var. *alpinum* and *Cassiope tetragona*. A number of species of mosses, including *Sphagnum teres*, form a sward about or sometimes below the axes of the dominants. The pH beneath one large tuft of *Sphagnum* was 5.4, the lowest found by Polunin this far north in Canada. Sphagna are not at all frequent in other habitats around Dundas.

2. At Pond Inlet (72°43′ N., 77°45′ W.) on the north shore of Baffin Island, tussocks 20 to 30 cm. (7.9 to 11.8 in.) high have developed in stagnant areas on the damp tundra on the plains. These form a variety of micro-habitats—wet tussocks, drier tussocks, tops of tussocks, sides of tussocks and spaces between tussocks. Mosses, including *Sphagnum warnstorfii* and species of *Drepancladus* as well as other genera are important everywhere. The soil is dark and humous only near the surface where it is slightly acid (pH 6.4).

3. Clyde "River" (70°27′ N., 68°35′ W.) is on the east shore of central Baffin Island. In some places on the undulating and comparatively low plain toward the head of a deep cove, *Sphagnum fuscum*, *S. capillaceum* var. *tenellum*, *S. girgensohnii*, *Aulacomnium palustre* and *A. turgidum* were much in evidence in the continuous investment among the higher plants. These

five species sometimes covered half of the area and formed tussocks up to 30 cm. (11.8 in.) in height.

4. Pangnirtung (66°6′ N., 65°30′ W.) is on a fiord extending from Cumberland Sound into southeastern Baffin Island. The country is rugged. In marshes bordering bodies of water in the lowlands near the sea, mosses often form a continuous investment among the taller phanerogams. The chief mosses in one small area were *Sphagnum capillaceum* var. *tenellum, S. teres* and species of *Aulacomnium, Calliergon, Drepanocladus, Oncophorus* and *Polytrichum.* The phanerogams were species of *Carex, Eriophorum, Arctagrostis, Dupontia, Luzula, Polygonum* and *Salix.*

5. Cape Dorset (64°10′ N., 76°30′ W.) is on Dorset Island which is very close to the west coast of the southern part of Baffin Island. In the marginal communities of some lakes and tarns there are open silty areas on which *Sphagnum fuscum* and species of *Calliergon* and *Scorpidium* grow along with algae (*Nostoc* spp. and *Sphaerella nivalis*). Among the vascular plants are species of *Carex, Juncus, Eriophorum, Deschampsia, Dupontia* and *Saxifraga.*

6. Chesterfield (63°20′ N., 90°42′ W.) is on the mainland on the west coast of Hudson Bay. In a small area of a marsh developed near the margin of a tarn, *Sphagnum squarrosum* grew with species of *Aulacomnium, Calliergon, Cinclidium, Dicranum, Drepanocladus, Meesea, Oncophorus* and *Polytrichum.* Among the vascular plants were species of *Carex, Salix, Potentilla, Arctagrostis, Luzula, Eriophorum* and *Equisetum.* This community was "young" and had little "grip" on the soil.

7. Wakeham Bay (61°36′ N., 71°57′ W.) is in northern Quebec on the southern shore of Hudson Strait. Variable marshes, generally of small extent, occur on comparatively rugged topography. In some places in the marshes a luxuriant growth of *Sphagnum capillaceum* var. *tenellum, S. squarrosum, S. compactum* and *S. lindbergii,* with species of *Calliergon, Drepanocladus, Oncophorus* and other genera of mosses form continuous mats. Polunin says: "*Sphagna* were more numerous than almost anywhere that I have seen in the north in these meridians, and with *Dicranum groenlandicum,* and *Aulacomnia* frequently assisted in the formation of tussocks". The angiosperms growing on these

tussocks were *Cassiope tetragona, Ledum palustre* var. *decumbens, Pedicularis hirsuta, Rubus chamaemorus* and *Vaccinium uliginosum* var. *alpinum.*

8. Port Burwell (60°25′ N., 64°52′ W.) is on Killinek Island in northern Labrador on the northeast coast of Ungava Bay. In discussing a small marsh in which the spermatophytes were carices, eriophora and species of other genera, Polunin says: "Occupying almost the whole of the area between the axes of these higher plants, and forming a rather smooth mat were a mixed assemblage of mosses of luxuriant growth". His list of the chief mosses includes *Sphagnum teres* and species of *Aulacomnium, Drepanocladus, Haplodon, Meesea* and *Paludella.*

SOUTHERN BOGS

WEST VIRGINIA. Thongh the occurrence of sphagnum bogs in Cranberry Glades in Pocohontas County had been known for many years, the first thorough investigation of them was made by Darlington (1943). He made field studies of this bog area over a period of 12 years, covering the geological, physiographic, climatic and biological factors which have influenced the development of the present forest, shrub, sedge, moss and lichen communities of the area. He also studied the successional trends which may indicate the future of the area.

He classifies the entire area of 600 acres as bog. The substrate of the whole region is acid (pH 3.8 to 6.2), and peat, varying in thickness from a few inches to 11 feet, covers most of it. The area is apparently level but actually has a drop of 45 feet in three miles. The deepest peat shows the following layers: sphagnum, two feet; sphagnum and sedge, three feet; sedge, two feet; algal ooze, four feet. Under this is three feet of marl, and then clay.

The general origin of the level area is attributed to surface erosion and differential rates of cutting by streams in rocks (limestones, sandstones, shales and conglomerates) having different degrees of resistance to cutting. Two uplifts of the area have occurred in geologic time. The origin of the depressions in which the deeper accumulations of peat have occurred is attributed largely to levee building by streams, especially near the confluence of two streams.

The general vegetational areas within the 600 acres are: shrubs, 400 acres; bog forest, 80 acres; sedges, mosses and lichens, 120 acres (in four "glades"). He recognizes the following plant communities: bog forest, *Alnus-Sambucus, Alnus-Viburnum, Carex-Sphagnum, Sphagnum-Vaccinium-Rynchospora,* and *Polytrichum-Cladonia.* All of the 600 acres except a part of the shrub community is partially covered with *Sphagnum.*

The *Polytrichum-Cladonia* association covers a total of 50 to 60 acres in two glades. The acidity of the substrate is high (pH 3.8) and the water table is three to six inches lower than in the communities surrounding it. There is a strong wind sweep through both areas of this association, and it may increase evaporation and transpiration, thus favoring growth of *Polytrichum* rather than of *Sphagnum.*

The air temperatures given are largely from records at Marlinton (elevation 2125 feet) over a period of 30 years. The elevation of Cranberry Glades is about 3400 feet. Hundreds of cold springs feed the streams in Cranberry Glades. Their temperature varies from 44° to 54° F. during the year. The temperature of the soil at a depth of six inches at a station in one of the glades was 37° F. on April 12 and 66° F. on August 12.

Among the successional trends which Darlington found by controlled field observations over a period of eight years or more are the following: *a*) in the *Polytrichum-Cladonia* association *Sphagnum* grew over *Polytrichum* under wet conditions, while the reverse was true under drier conditions; *b*) in this same association *Cladonia* grew rapidly when the weather was rainy and the water table high, but *Polytrichum* grew around and over *Cladonia* in drier weather; *c*) *Andromeda glaucophylla* spread rapidly in the *Polytrichum-Cladonia* association; *d*) *Gaultheria procumbens* spread rapidly in all directions from a clump of spruces in this same association; *e*) *Carex rostrata* encroached on a *Sphagnum-Vaccinium* community; *f*) shrubs encroached from all sides on one of the glade areas; *g*) the bog forest encroached on every side of one glade area. Darlington concludes that: "It is only a question of time until the shrubs will cover the whole 600 acres, gradually to be replaced by bog forest".

Rigg and Strausbaugh (1949) found that sphagnum bogs in various stages of development are common in seven counties in

the cool mountainous region of eastern West Virginia and occur occasionally in at least three other counties. The earliest stage studied was a fairly abundant growth of *Sphagnum imbricatum* var. *affine,* and perhaps other species, on the ground and on down timber in a fir (*Abies balsamea*) forest. This growth of *Sphagnum* spreads over an area of perhaps 100 acres at a general elevation of approximately 3500 feet near the base of Gaudineer Peak in Randolph County. The evidence that the *Sphagnum* is spreading is clear, but it has not formed any appreciable quantity of peat and there is no positive evidence that it will ever form a bog.

Later stages, ranging to the fairly mature stages in Cranberry Glades described by Darlington, were found by Rigg and Strausbaugh. Many of these occur in glades, some of them occupying the whole glade, some of them only a part of it. The term "glade", as used in West Virginia, designates level swampy areas whose vegetative cover may be entirely herbaceous or may consist also of shrubs or shrubs and trees. The term is not limited to areas in which *Sphagnum* grows.

The continued existence of depressions in which the West Virginia bogs examined have developed, outside of Cranberry Glades, is due to slow cutting by streams in the very resistant Pocono sandstone.

The origins of the sphagnum bog communities existing at present in West Virginia are accounted for in the main by the encroachment of *Sphagnum* and of the vascular plants which accompany it on other plant communities, and by the formation of sedge-*Sphagnum* mats over water.

Data for 1945 and 1946 from the U. S. Weather Bureau station at the Federal Prison, about half a mile from Cranberry Glades and at approximately the same level (3400 feet), indicate severe weather conditions. Rigg and Strausbaugh have summarized these conditions, especially those occurring during the growing season (April to August, inclusive).

Low temperatures, cloudy days, abundant precipitation and heavy snowfall are reported. During the growing season of 1946 there were 20 days (24 hour periods) during which the minimum temperature was 32° F. or lower. The distribution of these days by months was: April, 13; May, 4; June, 2; July, 0; August, 1.

The minimum temperatures for these months were: April, 16° F.; May, 28° F.; June, 29° F.; July, 39° F.; August, 32° F. During the growing season 24.1 per cent of the days were clear, 40.5 per cent were partly cloudy, and 35.3 per cent were cloudy. Precipitation (0.01 inch or more) fell on 32 per cent of these days. The total precipitation in inches and the maximum precipitation in any one 24-hour period were: April, 3.44, 1.33; May, 5.15, 1.15; June, 3.35, 1.26; July, 4.43, 1.95; August, 1.87, 0.51. The total snowfall during 1945 was 78.4 inches, and during 1946 it was 44.9 inches. Four inches of snow fell in April 1946 but only a trace in April 1945. The frost-free period for 1945 was 81 days (June 7 to August 27) and for 1946 it was 87 days.

These data do not necessarily indicate what the weather conditions were in the vicinity of other West Virginia sphagnum bogs, but the most extensive bogs and the most mature stages of succession occur in Cranberry Glades which are very close to the station at which the data were recorded. With one exception, all bog stages discussed are at elevations varying from 2560 to 3400 feet.

The bogs of West Virginia are more extensive and show much nearer approach to the climax stage (forest) than any known sphagnum bogs farther south. In general the species of bog plants, including *Sphagnum*, found in West Virginia bogs are more common north of this State than south of it.

The species of *Sphagnum* collected by Rigg and Strausbaugh in Cranberry Glades (determined by A. L. Andrews) were: *S. recurvum, S. palustre, S. capillaceum* var. *tenellum, S. magellanicum, S. imbricatum* var. *affine, S. recurvum* var. *tenue, S. squarrosum* and *S. girgensohnii*. The first five of these were also collected in one or more other bogs in the State. Darlington reports that *S. palustre* grows over most of the *Alnus-Viburnum* community and that the *Sphagnum* in the *Carex-Sphagnum* community is mostly *S. capillaceum* with some *S. palustre*.

FLORIDA. Davis (1946) gives general information in regard to bogs in which *Sphagnum* occurs. This is supplemented by a conversation with Davis in 1950. "*Sphagnum* moss and some sedges, grasses, heath shrubs, and other plants of more northern

bogs do occur in Florida, but these plants are most common in forest swamps and not in herbaceous marshes". Pine bogs or bays with *Sphagnum* and peat occur in Columbia, Baker, Lake and Polk counties. The first two counties are in the northern part of the State and the last two are in the central part. There are other northern counties with this type of bog.

The surface layer of growing and partly dead *Sphagnum* probably adds very little if anything to the accumulation of peat because of the high rate of oxidation of debris during most of the year, especially in dry seasons. When the water level is low this upper layer of material is subject to aeration and high temperatures (often above 80° F.), thus retarding *Sphagnum* growth and oxidizing dead parts. Much of the peat where *Sphagnum* occurs is formed from the roots of sedges and shrubs associated with the *Sphagnum*. These root systems are usually less exposed to aeration and do not oxidize so much as the moss does.

Murrill (1947) reports the following species of *Sphagnum* from Alachua County, Florida: *S. imbricatum, S. palustre, S. recurvum, S. strictum* and *S. subsecundum.* Harper (1910) gives in considerable detail accounts of the vegetation of peat areas in Florida.

TEXAS. Potzger and Tharp (1943, 1947) have recorded ecological features of Patschke Bog in Lee County, Texas. They say: "Deep bogs are not common in North America south of latitude 38°; for this reason the Patschke Bog is undoubtedly a rare find." The peat is 22 feet deep, consists almost entirely of finely divided wood fragments, is extremely black, and rests on fine reddish sand. The bog is now fed by springs and probably originated in a meander lake which was formed by the blocking of a small stream. The bog is in the Carrizo formation which is the lowest member of the Claiborne group near the middle of the Eocene system. The soils of the region are nine-tenths sand and one-tenth clay.

The bog was originally covered by a dense growth of *Ilex vomitoria, Myrica cerifera, Quercus nigra, Erianthus saccharoides, Panicum* spp., *Andropogon glomeratus,* many species of sedges, *Osmunda cinnamomea, O. regalis, Anchisica virginica,*

Lycopodium sp., *Sphagnum subsecundum, Rhexia marina* and *Pogonia ophioglossoidea.*

B. C. Tharp in a letter to Rigg in 1949 says: "*Sphagnum* occurs widely scattered in the wooded regions of eastern Texas, southwestward as far as Guadalupe County. In this extreme southwestern locality it still forms definite bogs. Some of these bogs embrace several acres of ground and from that others range down to a few square yards. Except in deep-shaded, wet-floored forests, I have never seen *Sphagnum* in Texas other than in bogs".

Rowell (1949) has described a small, shallow, seepage-fed bog of irregular shape lying between two sandy hills in Robertson County, Texas. He found *Mayaca aubleti, Sphagnum imbricatum* and *S. subsecundum* forming "dense mats on the surface wherever there is enough light for their growth". Among the species whose ecological roles he discusses are *Betula nigra, Sarracenia sledgei* and *Drosera annua.*

LOUISIANA. E. M. West in a letter to Rigg in 1949 says: "There are hundreds of acres in southeastern Louisiana mostly in the parishes (counties) of Tangipahoa, St. Tammany and Washington, in which *Sphagnum* is found, no great quantities anywhere. It forms thin layers over the ground surface, but to no great depths. No peat bogs are found anywhere in the State". Among the genera of vascular plants represented by one or more species in the same habitats as *Sphagnum* are *Drosera, Sarracenia, Gyrostachys, Dichromena, Ilex, Myrica, Vaccinium, Pinus, Quercus, Acer* and *Nyssa.*

ALABAMA. The work of Mohr (1901), based on collections made during "nearly forty years of sojourn and wanderings through the State", indicates that there were "bogs of peat mosses" in the coastal plain which borders the Gulf of Mexico in an area which supported " a rather uniform but magnificent vegetation of coniferous trees". In addition to *Sphagnum* he found species of *Sarracenia, Drosera* and *Pinguicula* in these bogs. He speaks of ample rainfall and the abundance of mosses in this coastal plain, and says: "Many of the declivities of the pine-clad hills and the depressions between them, of the exposed

swampy banks of the pine barren streams, and of the flats bordering the swamps in the plain, are covered with peat mosses (*Sphagnum*) of various kinds. The greater part of them are common from the Gulf to the Canadian lakes. Such are: *Sphagnum rufescens, S. recurvum, S. medium* and *S. imbricatum cristatum,* while others are confined to the subtropical region, namely *S. macrophyllum, S. microcarpum, S. ludovicianum, S. mobilense* and *S. mohrianum*".

Mohr lists 27 species of *Sphagnum* in the State, 18 of which are reported from Mobile County and one from Baldwin County. These two counties, as indicated by Mohr's map of floral areas, cover a considerable portion of the coastal plain area. Four of the species are reported from the Raccoon Mountains which are in the northeastern part of the State. Four species are reported from Lee County which, according to Mohr's map, is mountainous.

NORTH CAROLINA. H. L. Blomquist in a letter to Rigg in 1949 says: "There are in my opinion no extensive sphagnum bogs in North Carolina. However, there are numerous boggy areas in which some *Sphagnum* grows and these areas vary a good deal". He made a map showing six boggy areas in the western part of the State and five in the region from Durham County southward to Columbus County.

Blomquist (1938) has given distributional data for the species and varieties of *Sphagnum* occurring in the southeastern States of the United States.

TENNESSEE. Sharp (1939) lists the following species of *Sphagnum* in eastern Tennessee with habitats and comments: *S. magellanicum,* edge of spring, rare; *S. palustre,* wet soil, not common; *S. imbricatum,* wet soils, not common; *S. imbricatum* var. *affine,* more frequent than the species; *S. compactum,* wet soil and rocks, not common; *S. squarrosum,* wet rocks, rare, near the summit of Mt. Le Conte; *S. subsecundum,* wet soils and rocks, not common; *S. Pylaesii,* wet rocks, rare near the summit of Mt. Le Conte; *S. Girgensohnii,* wet rocks, rare, near the summit of Mt. Le Conte, the southern-most known station in North America; *S. capillaceum,* peat of "heath bald", rare; *S. quinquefarium,* wet rocks, not common; *S. tenerum,* wet rocks, very infrequent; *S. tabulare,* wet rocks, very infrequent.

In a letter to Rigg in 1950 Sharp says: "There are no large bogs in Tennessee and all the small ones are shallow. *Sphagnum* in the Smokies occurs in small raised clumps over seeps on bluffs usually. The small boggy areas are pretty much limited to the acid soils of the Cumberland Plateau and the Highland Rim country west of the Cumberlands. *Sphagnum* in small lots has been found in every physiographic region of the State".

T. F. Hall in a letter to Rigg in 1949 in regard to the Tennessee Valley says: "I would not consider *Sphagnum* common to this region as a whole, but good colonies have been observed in the eastern portion of the valley".

NEW WORK IN WELL KNOWN BOG REGIONS OF THE UNITED STATES

NEW JERSEY. Growing *Sphagnum* is found on the surface of many bogs, especially in the forest peat areas throughout the central and southern parts of the State (Waksman et al, 1943). Their profiles indicate that in the mountainous glaciated region in the extreme northern part of the State *Sphagnum* has taken some part in various stages of the development of comparatively deep bogs. It has not formed pure *Sphagnum* peat, but is mixed with *Carex, Phragmites,* forest and sedimentary peat. Even with these it has not extended entirely across any one profile. The peat strata in which sphagnum is a constituent vary in thickness from one to four feet at depths varying from the surface to 17 feet. In some bogs it has participated in the formation of two or even three strata of peat at different depths.

WASHINGTON. Two prairies in Grays Harbor County near the coast of the Pacific Ocean merit discussion in connection with the development of sphagnum bogs. The writer visited these in 1949. The word "prairie", as used in western Washington, designates open areas, treeless or bearing only scattered trees, in a generally forested region. The two prairies in Grays Harbor County are wet habitats on clay soil and are quite different from the relatively dry prairies on gravelly glacial outwash in Pierce and Thurston Counties.

Baker's Prairie (about 300 acres) is less than five miles from the ocean. It has one to two feet of black muck which lies on very porous grayish clay. On the surface of this muck *Sphag-*

num capillaceum and *S. papillosum* are growing vigorously in numerous flat patches; they also form some hummocks. No peat was found. The water table in August was about one foot below the surface. Local reports indicate that water about one foot deep stands on this prairie during the winter. The shrubs and trees on this prairie are mostly those that occur on sphagnum bogs in the region. The same is true of the herbs, and it is worthy of note that *Neprophyllidium cristagalli* (Menzies) Gilg., which is common in muskegs, bogs and other wet habitats in Alaska, here reaches its southern limit, so far as reported. The climate of this region is cold, wet and cloudy. The writer did not see any successional trends that would point to the development of this prairie into a sphagnum bog. Carlisle Prairie, which is also less than five miles from the ocean, is essentially like Baker's Prairie, and several other prairies apparently similar to these are mapped or locally reported in the region.

An extensive swamp forest, mainly coniferous, covering parts of ten sections, occurs on the Quinault Indian Reservation in Grays Harbor County a few miles east of these prairies. A surface growth of *Sphagnum* is common in this forest, and in some places there is fibrous peat of various depths down to eight feet. The forest has been much modified by logging and the building of logging roads. It would be difficult to predict the future vegetation of the area. The writer made a very general examination of it in 1949.

Further evidence of low temperatures of soil and air in sphagnum bogs was found by the writer (Rigg, 1947) in a bog on San Juan Island in the State of Washington. Soil and air thermograph records for a full year were obtained. Freezing temperatures of the air at a height of four feet over the bog occurred during every month of the growing season (April to August, inclusive) except August, when the minimum temperature was 34° F. The minimum for both April and May was 24° F., and for June and July, 28° F. The climate of the island outside of this bog is comparatively mild.

During the year of 1949 the writer did field work on the only known raised sphagnum bog in northeastern Washington. Other similar bogs in the same county (Pend Oreille) will be studied

during 1950 and details of all of them will be included in the forthcoming Bulletin of the State Division of Mines and Geology. The writer has also found that marl has played a large part in the development of bogs in the State of Washington, east of the Cascade Mountains. It has played a smaller part in bogs in western Washington. Diatomaceous earth also occurs in strata of varying thickness in bogs of eastern Washington, and some strata of this material occur in bogs of western Washington. Two occurrences of bog ore (bog iron) have been found in western Washington. Details of all of these will also be included in the forthcoming bulletin.

VOLCANIC ASH AS A TIME MARKER IN BOGS

Strata of volcanic ash, usually only a few inches thick, are common in sphagnum bogs and other peat deposits. In any region where the strata of volcanic ash in a number of bogs can be shown to have come from the same volcanic eruption, they furnish a basis for comparing the stages of development in the various bogs at that time. If the approximate date of the eruption can be fixed in geologic time the evidence becomes still more useful. The use of such data depends upon *a*) the certainty that the strata of volcanic ash in all of the bogs under consideration came from the same eruption, *b*) the determination of the volcano or at least the volcanic region from which the ash came, and *c*) the determination of the approximate geologic date of the eruption. Such data from sphagnum bogs and other peat deposits or sedimentary columns have been most generally used in connection with studies on fossil pollen as evidence on climate, plant succession and chronology, largely in postglacial times. If, however, the above criteria are satisfied the data become important in connection with the development of the bogs themselves.

Hansen (1947) bored many sedimentary columns in peat deposits of various types in the principal vegetational areas of Washington, Oregon, Idaho and British Columbia, and later (1949, 1949*a*) of Alberta. He says: "The occurrence of a stratum of volcanic ash in at least thirty postglacial sedimentary columns located in Washington, Idaho, and British Columbia records an eruption that dispersed ash over a wide area. The

source of this ash seems to be located in north central Washington, probably Glacier Peak where there is an abundance of pumice overlying the latest glacial drift (Waters, 1939)".

Hansen says also: "The greatest significance of the Washington volcanic ash is its tremendous value as a chronological indicator. Its occurrence in so many columns provides a common time marker and seems to correlate both chronology and forest succession over the entire region. It is dated about 6000 years (ago) as will be discussed later". Hansen's report represents over ten years of painstaking work. He quotes the literature fully.

Deevey (1948), in a review of Hansen's article, says: "H. P. Hansen has chosen for his research field one of the least understood regions of the country, and almost single handed has contrived to illuminate the postglacial history of an area nearly as large as Western Europe. His ten years of labor have been extraordinarily fruitful, and the resulting monograph will be welcomed in many quarters, as much for the path that it points as for what it contains". In commenting on Hansen's chapter on postglacial chronology Deevey says: "The principal objection is that the chronological tables are replete with absolute dates". Deevey also (1949) speaks of Hansen's "undue reliance on absolute dates".

It would be impossible within the scope of the present review to present Hansen's findings and conclusions fully or to go further into Deevey's comments on them in the two papers cited. Anyone interested in details should consult the three papers.

In 1949 the present writer and Marshall T. Huntting examined strata of volcanic ash in many bogs in both eastern and western Washington. They found white ash and yellow-brown ash. It is believed that the yellow-brown color is due to oxidation of iron compounds present in the ash. The yellow-brown ash was found most commonly in eastern Washington. In most cases the white ash formed a stratum at greater depths, while the yellow-brown stratum occurred at shallower depths, often near the surface. Occasionally, however, the two were found together at moderate depths. These findings do not in any way discredit the use of the deeper layers of white ash as markers of approximate and relative time; they merely raise new questions.

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